UNIVERSITY of TASMANIA

School of Land and Food

Enhancing long-chain omega-3 content in Australian lamb using genetics and diet.

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I dedicate this thesis to the memory of my brother George McDowall Bignell who tragically left this world on 15 May 2011.

"No experience is a cause of success or failure. We do not suffer from the shock of our experiences, so-called trauma - but we make out of them just what suits our purposes. We are self-determined by the meaning we give to our experiences, and it is almost a mistake to view particular experiences as the basis of our future life."

Alfred Adler 1931

Declaration

This is to certify that:

- The thesis contains no material which has been accepted for the award of other degree(s) in any tertiary institution
- To the best of my knowledge the thesis contains no materials previously published or written by any other person(s), except where due reference is made in the text
- The thesis may be made available for loan and limited copying in accordance with Copyright Act 1968
- All animals and procedures utilised in this study had the University of Tasmania Animal Ethics approval (A0009811) and were conducted in accordance with the 1993 Tasmanian Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes

C.W. Bignell
University of Tasmania
27 June 2016

Abstract

This thesis examined the effects of breed, sex, supplement and SNP marker on the levels of intramuscular long-chain omega-3 fatty acid content and meat quality traits in five Australian sheep breeds. Five hundred first-cross prime lambs sired by five genetically divergent breeds (Texel, White Suffolk, Dorset and East Friesian) under the same management conditions were utilised. The animals were grazed in a conventional broad acre sheep production system in southern Tasmania on improved and irrigated pastures. Adverse seasonal conditions necessitated the relocation of animals which had not reached slaughter weight to non-drought stressed pastures.

A supplementary feeding trial using 38 of the F_1 progeny representing the five breeds, two sexes (wethers and ewes), 2 supplementary feeds (canola or lupin) with two levels of supplementation (1% and 2% of body weight) was conducted over a 9-week duration. Overall, females had higher content of intramuscular long-chain omega-3 than males regardless of supplement type. The mean intramuscular eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) content of animals slaughtered at the commencement of the trial was only 7.5 mg/100 g. After supplementation, EPA + DHA content increased to 12.5 mg/100 g for canola meal and 14.3 mg/100 g with cracked lupin. Despite this remedial effect, supplementation still did not bring the claimable EPA + DHA content of Australian lamb up to the Food Standards Australia and New Zealand (FSANZ) claimable dietary source level of 30 mg/100 g.

The meat quality of 354 of the F_1 progeny was also investigated to test the impacts of single nucleotide polymorphic marker, sire breed, dietary supplementation and relocation to non-drought affected pastures on omega-3 fatty acid content and meat quality traits. Age at slaughter had an impact on fat scores and eye muscle shape, with younger animals having leaner subcutaneous fat and smaller eye muscles than older animals of the same carcass weight. The SNP markers tested did not have significant ($P>0.05$) effects on meat quality traits. The findings showed that as long as animals reach the required target liveweight and fat score before slaughter, rearing lambs on drought affected pasture, relocation or supplementation with canola or lupin meals to attempt to boost long-chain omega-3 content had no negative effect on the meat quality parameters. However, sire breed did have a significant effect on fat score and eye muscle measurement with the East Friesian lambs being leaner and having smaller eye muscle measurements but heavier muscle to bone yield.

Fatty acid profiles of the animals revealed an increase in intramuscular fat with time. Long-chain omega-3 fatty acid content closely reflected the quality of pasture on offer with only 7 mg/100 g EPA + DHA in the drought affected animals and this value doubled once animals were relocated to actively growing green pastures. Texel sired lambs had significantly lower ($P<0.05$) DHA content that other breeds.

The use of SNP markers to better understand the genetic variability in long-chain omega-3 fatty acid content and relationships with lipid synthesis and fat metabolism-related genes was also tested. The association between polymorphisms of the fatty acid binding proteins (FABP) and Delta-6 desaturase (FADS2) gene clusters was investigated. There was no significant ($P<0.05$) association between FADS2 or FABP4 and the intramuscular contents of EPA or DHA.

Microsatellite and SNP markers were utilised to genotype 79 sires from diverse Australian locations for the Myostatin gene. It was evident that the East Friesian and Texel breeds shared a common significant Guanine to Adenosine substitution (g+6723G>A) frequency of 0.63, thus suggesting a common phylogenetic origin.

As an overall outcome for this thesis, Australian sheep meat producers can better understand the significance of supplementation with canola or lupin, quality of feed, sex, genotype and breed on the long-chain omega-3 content of sheep meat. Future research into the fatty acid profiling of various fodder and pastures commonly used in sheep grazing systems over time is of strong merit, as is the trialling and potential use of a wider range of supplements. The high levels of variation observed and the failure to date to reach claimable source (30 mg/100 g) and ultimately good source (60 mg/100 g) levels are still of concern. These two claimable levels should remain research and production targets, if Australian lamb is to be considered a reliable dietary source of long-chain omega-3 for human consumers.

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List of Abbreviations

ABARE	Australian Bureau of Agricultural & Resource Economics & Sciences
ADG	Average Daily Gain
ALA	Alpha-linolenic acid
AOCS	American Oil Chemists Society
ARA	Arachidonic acid
C_{20}	Denoting the number of carbons in a fatty acid
DGLA	Dihomo-gamma-linolenic acid
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
EBV	Estimated Breeding Value
EMA	*Longissimus dorsi* area
EMH	*Longissimus dorsi* height
EPA	Eicosapentaenoic acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FSANZ	Food Standards Australia and New Zealand
GC	Gas Chromatography
GR Fat	Subcutaneous fat layer at the 12^{th} and 13^{th} rib of a carcass
GLA	Gamma-linolenic acid

INF	Information Nucleus Flock
IMF	Intramuscular Fat
IMFC	Intramuscular Fat Content
LC	Long-chain (≥C_{20})
LC-PUFA	Long-chain polyunsaturated fatty acid(s)
MLA	Meat and Livestock Australia
MUFA	Monounsaturated fatty acid(s)
PUFA	Polyunsaturated fatty acid(s)
RDI	Recommended Daily Intake
SDA	Stearidonic acid
SFA	Saturated fatty acid(s)
SNP	Single Nucleotide Polymorphic GDF8
TLE	Total Lipid Extract
ω3	Omega-3
ω6	Omega-6

List of Publications

During the course of this study a number of publications and public presentations have been made which are based on the work presented in this thesis. They are listed below for future reference and attached in the appendices.

Journal Articles

Bignell, C. W., Malau-Aduli, A. E. O., Nichols, P. D., McCulloch, R. & Kijas, J. W. (2010). East Friesian sheep carry a Myostatin allele known to cause muscle hypertrophy in other breeds. *Animal Genetics* 41(4): 445-446

Wijesundera, C., Kitessa, S., Abeywardena, M., Bignell, W. & Nichols, P. D. (2011). Long-chain omega-3 oils: Current and future supplies, food and feed applications, and stability. *Lipid Technology* 23(3): 55-58

Malau-Aduli, A.E.O., Ranson, C.F., Bignell, C.W. (2009a). Wool quality and growth traits of Tasmanian pasture-fed crossbred lambs and relationships with plasma metabolites. J. Anim. Sci., 87 (E-Suppl 2), 499.

Malau-Aduli, A.E.O., Walker, R.E., Bignell, C.W. (2009). Prediction of wool fibre diameter from protein and metabolisable energy digestibility coefficients in crossbred sheep. J. Anim. Sci., 87 (E-Suppl 2), 498.

Conferences and Presentations

Bignell, C., Nichols, PD, Malau-Aduli, AEO, Kijas, J.W. (2009). Influence of lupins and canola supplement on short loin fatty acid profiles within genetically divergent first cross merino lambs. *28th Congress of the International Society for Fat Research* Sydney.

Bignell, C. W., Nichols, P. D., Malau-Aduli, A. E. O., Kijas, J. W. & McCulloch R. (2011) Intramuscular long-chain omega 3 content in Australian lamb: Drought effect, genomics and dietary improvement strategies*. Australasian Section of the American Oil Chemists Society* Adelaide.

Malau-Aduli, A.E.O., Walker, R.E., Bignell, C.W. (2009). Variation in sire genetics is an irrelevant determinant of digestibility in supplemented crossbred sheep. In: Y. Chilliard, F. Glasser, Y. Faulconnier, F. Bocquier, I. Veissier, M. Doreau (Editors), Wageningen Academic Publishers, The Netherlands. *Proceedings of the XIth International Symposium on Ruminant Physiology*, 6-9 September 2009, Clermont-Ferrand, France, 11, 278-279.

Malau-Aduli, A.E.O., Walker, R.E., Ranson, C.F., Sykes, J.M., Bignell, C.W. (2009).Nutrition-genetics interaction in nutrient utilization of canola and lupins by Australian sheep: Prediction of wool fibre diameter. In: D. Sauvant (Editor) *Proceedings of the 7th International Workshop on Modelling Nutrition, Digestion and Utilization in Farm Animals*, 10-12 September 2009, AgroParisTech, Paris, France., 7, 50.

Malau-Aduli, A.E.O., Sykes, J.M., Bignell, C.W. (2009). Influence of lupins and canola supplements on plasma amino acids, wool fibre diameter and liveweight in genetically divergent first cross Merino lambs. *Proceedings of the World Congress on Oils and Fats & 28th International Society for Fats Research Congress*, 27-30 September 2009, Convention & Exhibition Centre, Sydney, Australia., 28, 63.

Awards

2009 – AusBiotech-GSK Student Excellence Awards – State Winner

2009 – American Oil Chemists Society, Australasian Section Student Travel award for outstanding academic achievement.

2010 – Premier's Young Achiever of the year.

Fonterra Agriculture Young Achiever Award.

2011 – American Oil Chemists Society, Australasian Section Bryce Bell Student Award for Best Oral Presentation.

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Statement of Co-Authorship

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Located in Chapter 6. Candidate was the primary author, author 3 contributed to the idea and development of molecular markers chosen in the experiment. Authors 1 and 2 assisted with the refinement and presentation of the short communication.

We the undersigned agree with the above stated “proportion of work undertaken” for the above published peer-reviewed paper contributing to this thesis:

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Chapter 1

General Introduction

Overview

The scope for improving the long-chain (≥C_{20}) omega-3 polyunsaturated fatty acid (PUFA) content in Australian lamb via genetic selection and dietary manipulation is addressed in this thesis. The Australian population consumes on average 10 kg of sheep meat per annum and the potential to boost population health via consumption of a more healthy red meat is of great interest to Australian lamb producers and an increasingly heath aware consumer base (MLA, 2014). This introductory chapter explains the research rationale, justification, hypotheses, objectives and the potential impacts of drought, genetics and supplementary diet on long-chain omega-3 content in Australian lamb.

This study examined the first cross progeny of five terminal sires (Texel, East Friesian, White Suffolk, Coopworth and Dorset) from joining with Merino dams raised under typical extensive Australian sheep meat production system. The study was undertaken during a severe drought (BOM, 2008) characterised by decreased feed availability and negative production impact on animal performance and long-chain omega-3 content in lamb

Dietary supplementation with canola and cracked lupin meal in a feeding trial was conducted to investigate possible mitigation techniques to ameliorate the negative impacts of drought on animal performance and long-chain omega-3 content in lamb. The potential for genetic markers to predict long-chain omega-3 content was also

investigated and the detection of the growth differentiation factor 8 (GDF8 or Myostatin) mutation in East Friesian progeny provided conclusive evidence for shared genes and phylogenetic origin with the Texel sheep breed.

Factors driving long-chain omega-3 consumption

There has been considerable interest by health professionals, research bodies and consumers around the scientifically proven health benefits of long-chain omega-3s. There is strong evidence that demonstrates that a regular intake of long-chain omega-3 fatty acids can reduce the incidence of cardiovascular and inflammatory diseases such as arthritis and improve mental function and health (Gogus and Smith, 2010; Clayton, 2014). The key fatty acids identified as driving these benefits are the long-chain omega-3 polyunsaturated fatty acids - eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3). These are dietary indispensable acids which humans generally cannot synthesize and their major source in the human diet has traditionally been from wild caught oily fish such as mackerel and tuna and more recently aquaculture based seafood.

To obtain and maintain the health benefits of long-chain omega-3, traditionally two servings of fatty fish per week was recommended (Baghurst, 2006). The Australian National Health and Medical Research Council proposed use of the recommended daily intake (RDI) model and suggest consuming 410 mg/d for women and 630 mg/d for men as the minimum RDI (Baghurst, 2006). Many Eastern countries with a traditionally high fish intake such as Japan achieve this relatively easily, however, countries such as Australia and the United States struggle to meet this RDI and it is estimated Australian's on average only achieve 140g / day (Meyer et al., 2003). Surveys of the Australian diet have shown that up to 70% of the long-chain omega-3 in our diets is seafood derived, 20% from meats and 6% from eggs (Meyer et al.,

2003; Howe et al., 2006). The majority of seafood consumed in Australia is imported and not wild caught, which often has significantly different fatty acid profiles to its wild counterpart and compounds the problem of the majority of Australians not meeting the long-chain omega-3 RDI (Howe et al., 2006; Nichols et al., 2010).

Aquaculture has been the fastest growing food producing sector in the world (Tacon, 2003). Farmed seafood requires supplementation with wild harvest derived fish oils rich in EPA + DHA to offer the health benefits expected by educated consumers. As a result, the demand for long-chain omega-3 rich oils for aquafeeds has increased significantly in recent decades. However, competing industries such as the nutraceutical, pharmaceutical and agricultural sectors have also increased their demand for the wild harvest fish oil resource placing significant pressures on the price and availability of EPA + DHA rich fish oils (Nichols et al., 2010).

The principal fish oil resource is wild caught small pelagic fishes such as anchovies which globally have experienced intensified fishing efforts (Schwartzlose et al., 1999; Sanchez et al., 2000). However, global catches have reached a plateau suggesting the potential to meet the increased future demand is limited (Naylor et al., 2000; Black, 2008). Alongside wild fisheries biomass exploitation having peaked, the ecological impacts upon the fish species targeted has also led to consumer demands for other foods containing ‘good sources’ (defined as 60 mg/100 g) of long-chain omega-3 fatty acids from sources other than seafood (Pullin et al., 2007; Naylor et al., 2000).

These two factors of varied long-chain omega-3 content in seafood and the failure of the typical Australian diet to meet the long-chain omega-3 RDI has emphasized the need for methods to expand the availability of foodstuff containing the long-chain omega-3 EPA and DHA (Murphy et al., 2007). Fortification of many every day food

items such as biscuits, milk, bread and juice has been one approach to boost daily intake of long-chain omega-3 (Murphy et al., 2007; Howe et al., 2006). In many cases, the long-chain omega-3 found in such foods is derived from the wild harvest fisheries, although other sources such as algal-derived oils are also used. The higher costs of manufacture and the challenges of algal oil being higher in price than fish oils coupled with the limited industrial scale production capability has restricted the scale of uptake of algal oil for use in processed and fortified foods (Ward and Singh, 2005). Hence fortification of processed foods is one component towards a solution for alternative dietary sources of long-chain omega-3 and enhanced population health.

Absolute values of omega-3 intake is a valuable measure for healthy eating, but it has also been recognised that the ratio of omega-3 to omega-6 fatty acids (ω3/ω6) in foods is also of importance (Simopoulos, 2002; Goodstine *et al.*, 2003; Clayton, 2014). The western diet has a strong influence of oils and spreads rich in monounsaturated fatty acids (MUFA) and also rich in omega-6 fatty acids leading to extremely high ω6/ω3 ratios. A high level of omega-6 intake is associated with cardiovascular disease, cancer, inflammatory and autoimmune disease in humans (Simopoulos, 2002). The optimal ratio of ω3/ω6 is considered to be anywhere from 1 to 0.25, but for the modern Australian diet, it is estimated that the ratio is closer to 0.13 ω3/ω6, highlighting the need to increase the nation's intake of omega-3 fatty acids (Howe et al., 2006; Simopoulos, 2002; Goodstine et al., 2003).

The increased demand for omega-3 has led to many products being marketed as a source of dietary omega-3 without distinction between long-chain and shorter chain ($\leqslant$C18) omega-3 to capitalise on strong consumer understanding of the perceived benefits of omega-3 fatty acids (Verbeke et al., 2009; Turchini et al., 2012).

Terrestrial sources of omega-3 such as walnut and chia seed are rich sources of the shorter chain omega-3 acid alpha-linolenic acid (ALA). However, terrestrial sources generally contain minimal if any, long-chain omega-3. ALA does offer significant health benefits, but the key health benefits and the presently limited dietary intake which both need addressing is with respect to the long-chain omega-3 fatty acids EPA + DHA.

The human body and many animals have the ability to elongate the shorter chain fatty acids such as ALA to long-chain fatty acids (EPA + DHA), but the conversion is extremely inefficient, with women having the highest recorded efficiency of up to only 5.5% (Burdge, 2006). Studies have shown the importance of having an ample dietary intake of the precursor shorter chain omega-3 fatty acids, but demand for conversion is effected by many aspects including developmental age and overall health status of the individual (Cunnane, 2000). Given the very low conversion rate and variability of ALA to EPA, and in particular to DHA, the potential to increase human intake of long-chain omega-3 via ingestion of the shorter chain omega-3 is very limited in potential (Burdge, 2006).

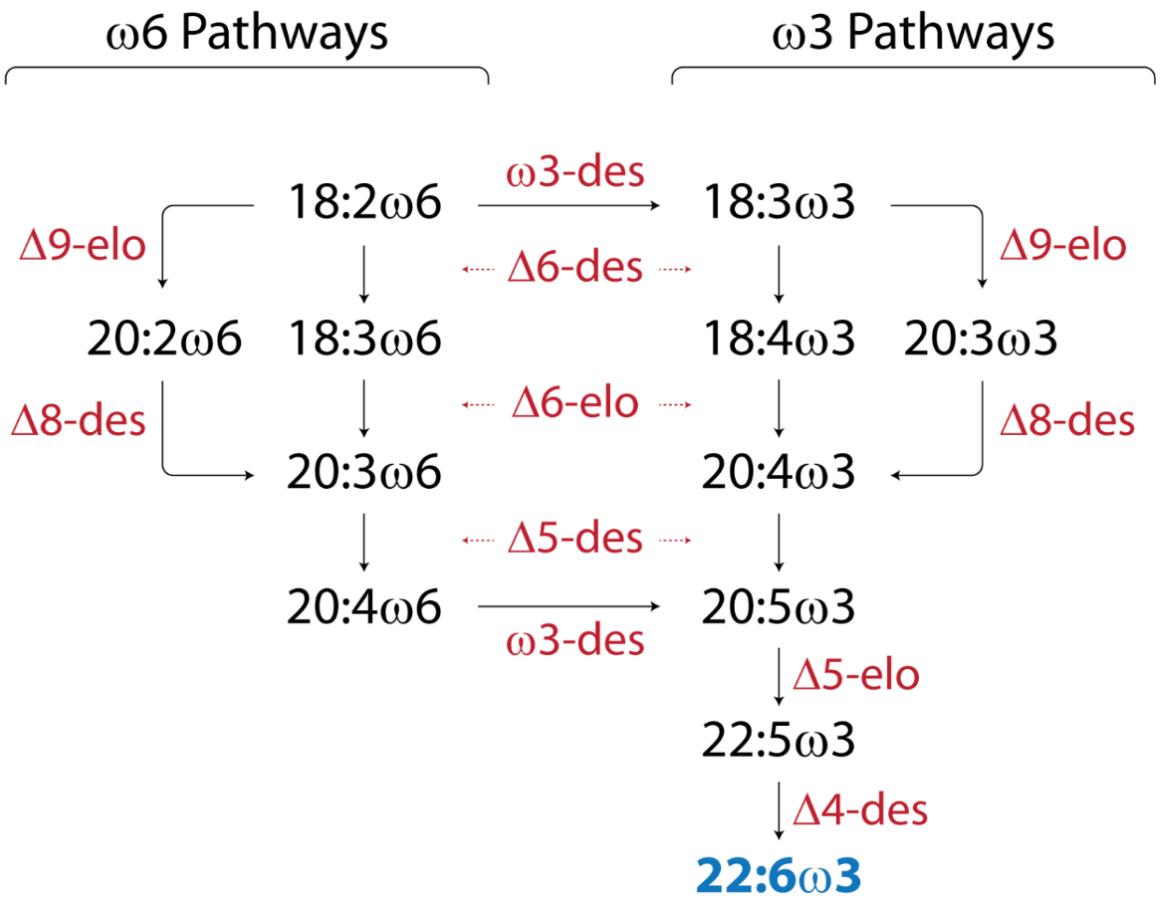

Figure 1.1 The general dietary indispensable fatty acid desaturation/elongation pathway. Adapted from Tocher (2003).

Meat has traditionally been considered a significant source of dietary saturated fatty acids (SFA) and MUFA in the Australian diet. Whilst it does contribute a large proportion of dietary SFA, it is also contributing up to 20% of the long-chain omega-3 Australians are consuming (Howe *et al.*, 2006). Changes in consumer behaviour and demand for leaner meat has led to a change in the meat being sold in Australia and its consumption, in particular with lamb (Williams and Droulez, 2010). Australians are now consuming leaner cuts of lamb with the SFA rich subcutaneous fat trimmed and removed, and a recent study has shown lamb is no longer such a major dietary source of SFA (Williams and Droulez, 2010; Pethick *et al.*, 2011).

Lush, unstressed green pasture is rich in ALA and grass and a key feed source in Australian sheep meat systems along-side specialty fodder crops such as rape, lucerne and dual purpose cereals. It has been well documented that grass-fed red meat contains a healthier balance of fatty acids compared to grain-fed animals, and that grass-fed animals contain varied levels of the key long-chain omega-3 fatty acids EPA + DHA derived from the desaturation of ALA (Clayton, 2014). Sheep have the ability to convert ALA to long-chain omega-3 including the intermediary fatty acid docosapentaenoic acid (DPA, 22:5ω3) and several studies have shown huge levels of variation both within and across breeds which needs careful consideration and management when assessing potential long-chain omega-3 content (Aurousseau *et al.*, 2004; Aurousseau *et al.*, 2007a; Carvalho and Medeiros, 2010; Kitessa *et al.*, 2010; Kitessa *et al.*, 2001; Komprda *et al.*, 2012).

The variation in long-chain omega-3 content in animal meat products can be mitigated via supplementation using rumen protected fish oils and such application has been demonstrated in sheep (Kitessa *et al.*, 2001). However, the process has never been adopted in commercial operations for various reasons; this has led to a focus on improving the ALA content of feeds on offer and genetic improvement being more favourable approaches. The variation of ALA in pasture species is a significant source of the variation of long-chain omega-3 content in animal meat products. Dewhurst *et al.* (2001) found that the level of ALA varied by a factor of 12 within 3 species of ryegrass under glass house conditions. Field trials have found that biotic and abiotic stresses have a greater effect than plant genetics on fatty acid levels. ALA levels in grass are reported to drop over summer as the pasture dries and studies using summer pastures and dry hay have shown a decrease in content of

intramuscular ALA and long-chain omega-3 in lamb meat as a result (Aurousseau *et al.*, 2007e; Dierking, 2008; Elgersma *et al.*, 2003; Kitessa *et al.*, 2010).

The PUFA in sheep meat is predominately stored in the phospholipid fraction of the meat and only 8% is found in the triacylglycerols meaning the majority of the beneficial long-chain omega-3 is within the muscle being consumed and benefiting the consumer (Ponnampalam *et al.*, 2014; Williams and Droulez, 2010). As animals age their total lipid content increases and the concentration of SFA and MUFA also increases, while PUFA stays relatively stable (Raes *et al.*, 2004). The production of lamb, the most commonly consumed sheep meat in Australia, is based on animals which are young and not reached full maturity (15 months) and such animals therefore still have a favourable PUFA content relative to SFA and MFA and hence a healthier fatty acid profile.

The regions of Australia producing livestock are highly varied and the impact of this variation is just one further factor having an impact on the long-chain omega-3 content of lamb. Lambs being grown for meat consumption require high quality nutrition from birth to slaughter to maintain sufficient growth rates and expected meat quality. As a result, the majority of sheep meat production regions have been high rainfall areas with sufficient natural rainfall to have extended growing seasons or having irrigation available to fill feed gaps in natural pasture growth variation. Supplementation with ALA rich foodstuffs has been demonstrated to be beneficial to fatty acid profiles, however, supplementation with grains can have a negative impact. Grains acidify the rumen and decrease its efficiency and increase the biohydrogenation of the PUFA and MUFA to SFA, ultimately decreasing the healthy eating qualities of the meat (Demeyer and Doreau, 1999).

Given the constraint on the global resource for long-chain omega-3 fish oils and the need to find dietary alternatives to boost population health, Australian lamb meat is a strong candidate. The ability of sheep to convert the ALA rich principal grass feed into EPA, DPA & DHA, coupled with the lower SFA and MUFA ratios of younger animals and a consumer change towards leaner, heavily trimmed cuts of lambs, all work towards achieving the goal of providing an alternative dietary source of long-chain omega-3. However, there is a clear need to help reduce the high levels of variability in long-chain omega-3 content within breeds and individual animals, production zone variation and also to mitigate the negative impacts of supplementation. Progress on these and other areas will assist towards achieving cost effective sheep meat consistently meeting the 30 mg / 100 g “source” claims, although at the present time further investigation is needed to push towards the ultimate goal of achieving the 60 mg / 100 g “good source”.

Thesis aims and hypotheses

The overall concentration of long-chain omega-3 in lamb can be affected by many factors including diet, breed age and sex. Therefore to increase and improve the long-chain omega-3 content, a multifaceted approach is required for understanding the effects of sire breed, age, sex, type of feed and supplementation. To address the aims of this study, the following approaches were adopted:

- To quantify the variation of long-chain omega-3 fatty acids in five first cross sheep meat breeds reared on pastures either affected by drought or irrigated and supplemented.
- Understand the effects on long-chain omega-3 of finishing lambs with LA and ALA containing supplements - canola meal and lupin - in a 9 week feeding trial.
- Investigation of the potential use of single nucleotide polymorphic (SNP) molecular markers as a breeding tool to predict long-chain omega-3 content association with sire breed and supplement.
- To examine and detect the growth differentiation factor 8 (GDF8 or Myostatin) mutations in the flock.

Each of the above approaches formed the basis of the hypotheses tested in the following chapters of this thesis:

Chapter 2 examines the hypothesis that supplementing animals from a low intake of ALA (drought pasture) with LA and ALA containing canola meal and lupin meal would improve EPA + DHA content in Australian lambs to dietary source levels.

Chapter 3 hypothesised that improved long-chain omega-3 content in lamb meat will not have a negative impact on meat quality traits.

Chapter 4 tested the hypothesis that drought affected pasture will have a negative impact on long-chain omega-3 content and relocation to improved lush green pasture will improve long-chain omega-3 content.

Chapter 5 investigated single gene association between SNP markers and long-chain omega 3 will not be significantly influenced by sire breed or supplement.

Chapter 6 tested the hypothesis that a significant detection of the GDF8 mutation will align shared phylogenetic source of origin with other known sheep breeds.

Chapter 7 discusses and summarises the overall research direction of enhancing the long-chain omega-3 content in sheep meat and potential areas for further investigation.

References

Aurousseau, B., Bauchart, D., Calichon, E., Micol, D. & Priolo, A. (2004). Effect of grass or concentrate feeding systems and rate of growth on triglyceride and phospholipid and their fatty acids in the M-longissimus thoracis of lambs. *Meat Science* 66(3): 531-541.

Aurousseau, B., Bauchart, D., Faure, X., Galot, A. L., Prache, S., Micol, D. & Priolo, A. (2007a). Indoor fattening of lambs raised on pasture. Part 1: Influence of stall finishing duration on lipid classes and fatty acids in the longissimus thoracis muscle. *Meat Science* 76(2): 241-252.

Aurousseau, B., Bauchart, D., Faure, X., Galot, A. L., Prache, S., Micol, D. & Priolo, A. (2007b). Indoor fattening of lambs raised on pasture: (1) Influence of stall finishing duration on lipid classes and fatty acids in the longissimus thoracis muscle. *Meat Science* 76(2): 241-252.

Baghurst, K. (2006).Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes. Australia.233-236

Black, K. D. (2008).Environmental Aspects of Aquaculture. In *Aquaculture, Innovation and Social Transformation*, Vol. 17, 97-113 (Eds K. Culver and D. Castle). Dordrecht: Springer.

BOM (2008). Long-term rainfall deficiencies continue in southern Australia while wet conditions dominate the north. . In *Special Climate Statement 16*, 9 (Ed N. C. Centre). 700 Colins St Docklands: Bureau of Meteorology.

Burdge, G. C. (2006). Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukotrienes and Essential Fatty Acids* 75(3): 161-168.

Carvalho, S. & Medeiros, L. M. (2010). Carcass characteristics and meat composition of lambs finished in feedlot with diets containing different levels of energy. *Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science* 39(6): 1295-1302.

Clayton, E. H. (2014).Graham Centre Monograph No. 4: Long-chain omega-3 polyunsaturated fatty acids in ruminant nutrition: benefits to animals and humans. (Eds T. Nugent and C. Nicholls). Wagga Wagga NSW: NSW Department of Primary Industries.

Cunnane, S. C. (2000). The conditional nature of the dietary need for polyunsaturates: a proposal to reclassify 'essential fatty acids' as 'conditionally-indispensable' or conditionally-dispensable' fatty acids. *British Journal of Nutrition* 84(6): 803-812.

Demeyer, D. & Doreau, M. (1999). Targets and procedures for altering ruminant meat and milk lipids. *Proceedings of the Nutrition Society* 58(3): 593-607.

Dewhurst, R. J., Scollan, N. D., Youell, S. J., Tweed, J. K. S. & Humphreys, M. O. (2001). Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. *Grass and Forage Science* 56(1): 68-74.

Dierking, R. M. (2008).Fatty acid variation between forage species and within populations populations and fatty acid content of beef finished on pasture with different forrage species. In *Division of Plant Sciences*, Vol. Master of Science, 84: University of Missouri.

Elgersma, A., Ellen, G., Horst, H. v. d., Muuse, B. G., Boer, H. & Tamminga, S. (2003). Influence of cultivar and cutting date on the fatty acid composition of perennial ryegrass (Lolium perenne L.). *Grass and Forage Science* 58(3): 323-331.

Gogus, U. & Smith, C. (2010). n-3 Omega fatty acids: a review of current knowledge. *International Journal of Food Science and Technology* 45(3): 417-436.

Goodstine, S. L., Zheng, T. Z., Holford, T. R., Ward, B. A., Carter, D., Owens, P. H. & Mayne, S. T. (2003). Dietary (n-3)/(n-6) fatty acid ratio: Possible relationship to premenopausal but not postmenopausal breast cancer risk in US women. *Journal of Nutrition* 133(5): 1409-1414.

Howe, P., Meyer, B., Record, S. & Baghurst, K. (2006). Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition* 22(1): 47-53.

Kitessa, S., Liu, S. M., Briegel, J., Pethick, D., Gardner, G., Ferguson, M., Allingham, P., Nattrass, G., McDonagh, M., Ponnampalam, E. & Hopkins, D. (2010). Effects of intensive or pasture finishing in spring and linseed supplementation in autumn on the omega-3 content of lamb meat and its carcass distribution. *Animal Production Science* 50(2): 130-137.

Kitessa, S. M., Gulati, S. K., Ashes, J. R., Scott, T. W. & Fleck, E. (2001). Effect of feeding tuna oil supplement protected against hydrogenation in the rumen on growth and n-3 fatty acid content of lamb fat and muscle. *Australian Journal of Agricultural Research* 52(4): 433-437.

Komprda, T., Kuchtik, J., Jarosova, A., Drackova, E., Zemanek, L. & Filipcik, B. (2012). Meat quality characteristics of lambs of three organically raised breeds. *Meat Science* 91(4): 499-505.

Meyer, B. J., Mann, N. J., Lewis, J. L., Milligan, G. C., Sinclair, A. J. & Howe, P. R. C. (2003). Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipids* 38(4): 391-398.

MLA (2014).Australian Sheep Meat Industry Overview. In *Australian Sheep Meat Industry Projections 2014*: Meat and Livestock Australia.

Murphy, K. J., Meyer, B. J., Mori, T. A., Burke, V., Mansour, J., Patch, C. S., Tapsell, L. C., Noakes, M., Clifton, P. A., Barden, A., Puddey, I. B., Beilin, L. J. & Howe, P. R. C. (2007). Impact of foods enriched with n-3 long-chain polyunsaturated fatty acids on erythrocyte n-3 levels and cardiovascular risk factors. *British Journal of Nutrition* 97(4): 749-757.

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C. M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature* 405(6790): 1017-1024.

Nichols, P. D., Petrie, J. & Surinder, S. (2010). Long-chain omega-3 oils - an update on sustainable sources. *Nutrients* 2(6): 572-585.

Pethick, D. W., Ball, A. J., Banks, R. G. & Hocquette, J. F. (2011). Current and future issues facing red meat quality in a competitive market and how to manage continuous improvement. *Animal Production Science* 51(1): 13-18.

Ponnampalam, E. N., Butler, K. L., Pearce, K. M., Mortimer, S. I., Pethick, D. W., Ball, A. J. & Hopkins, D. L. (2014). Sources of variation of health claimable long chain omega-3 fatty acids in meat from Australian lamb slaughtered at similar weights. *Meat Science* 96(2, Part B): 1095-1103.

Pullin, R. S. V., Froese, R. & Pauly, D. (2007). *Indicators for the sustainability of aquaculture.* Dordrecht: Springer. 53-72.

Raes, K., De Smet, S. & Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. *Animal Feed Science and Technology* 113(1-4): 199-221.

Sanchez, G., Calienes, R. & Zuta, S. (2000). The 1997-98 El Nino and its effects on the coastal marine ecosystem off Peru. *California Cooperative Oceanic Fisheries Investigations Reports* 41: 62-86.

Schwartzlose, R. A., Alheit, J., Bakun, A., Baumgartner, T. R., Cloete, R., Crawford, R. J. M., Fletcher, W. J., Green-Ruiz, Y., Hagen, E., Kawasaki, T., Lluch-Belda, D., Lluch-Cota, S. E., MacCall, A. D., Matsuura, Y., Nevarez-Martinez, M. O., Parrish, R. H., Roy, C., Serra, R., Shust, K. V., Ward, M. N. & Zuzunaga, J. Z. (1999). Worldwide large-scale fluctuations of sardine and anchovy populations. *South African Journal of Marine Science-Suid-Afrikaanse Tydskrif Vir Seewetenskap* 21: 289-347.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy* 56(8): 365-379.

Tacon, A. G. J. (2003). *Growing requirements for fish meals and fish oils.* Fairbanks: Alaska Sea Grant Coll Program.

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science* 11(2): 107-184.

Turchini, G. M., Nichols, P. D., Barrow, C. & Sinclair, A. J. (2012). Jumping on the omega-3 bandwagon: distinguishing the role of long-chain and short-chain omega-3 fatty acids. *Crit Rev Food Sci Nutr* 52(9): 795-803.

Verbeke, W., Scholderer, J. & Lahteenmaki, L. (2009). Consumer appeal of nutrition and health claims in three existing product concepts. *Appetite* 52(3): 684-692.

Ward, O. P. & Singh, A. (2005). Omega-3/6 fatty acids: Alternative sources of production. *Process Biochemistry* 40(12): 3627-3652.

Williams, P. & Droulez, V. (2010). Australian red meat consumption - implications of changes over 20 years on nutrient composition. *Food Australia* 62(3): 87-94.

Chapter 2

Supplementing lamb with canola meal and cracked lupin to remediate the negative effect of severe drought on long-chain omega-3 content in Australian lamb.

Abstract

Supplementary feeding is used to maintain livestock during limited pasture growth (drought) and this experiment investigated the potential of canola meal and cracked lupins to remediate the decrease in long-chain omega-3 (LC omega-3) content of drought affected lambs. The experiment utilised 38 first cross Merino lambs in a 5 x 2 x 2 x 2 factorial design, incorporating five commonly used sire breeds in Australia (Texel, Dorset, East Friesian, Coopworth and White Suffolk), 2 supplementary feeds (canola meal and lupin), two genders (wethers and ewes) and 2 levels of supplementation (1% and 2% of body weight). Animals were housed in individual metabolic crates for the 9-week feeding trial comprising 3 weeks of adjustment and 6 weeks of data collection. Overall, the mean total intramuscular fat (IMF) was not significantly influenced by level of supplementation, sex, sire breed, or supplement. Canola supplemented prime lambs had 3.7 ± 0.3% IMF versus 3.4 ± 0.2% in lupin supplemented lambs. However, there were significant interactions between sire breed and supplement whereby lambs sired by White Suffolk supplemented with lupins had the lowest IMF of 2.7 ± 0.6% while Coopworth and East Friesian sired lambs fed lupins and canola had the highest IMF contents of 4.3 ± 0.6% and 4.3 ± 0.8% respectively. Sire breed was a significant source of variation for the fatty acids 18:2ω6 (P=0.04) and 20:3ω6 (P=0.03) with Texel and White Suffolk progeny having lower mean concentrations (92.4 ± 9.2 mg/100 g & 92.6 ± 11.5 mg/100 g

respectively) and Coopworth having the highest (113.8 ± 16.2 mg/100 g). Sex differences were significant as females deposited more intramuscular eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) and docosapentaenoic acid (DPA, 22:5ω3) than males irrespective of supplement. Overall, females had higher content of intramuscular long-chain omega-3 than males regardless of supplement type. The mean intramuscular EPA + DHA content nearly doubled in comparison to drought affected animals from the same genetic flock (7.5 mg/100 g). After supplementation with canola, EPA + DHA content increased to 12.5 mg/100 g and 14.3 mg/100 g with cracked lupin. Despite this remedial effect, supplementation still did not bring the claimable EPA + DHA content up to FSANZ claimable dietary "source" levels of 30 mg/100 g. This finding shows that despite supplementation with ALA rich canola meal or cracked lupin, drought has a negative effect, with respect to long-chain omega-3 content, on the healthy eating characteristics of Australian lamb.

Key Words: lamb, omega-3, polyunsaturated, fatty acid, canola, lupin, sheep, ovine, drought, supplementation.

Introduction

Australia is one of the world's largest livestock producers, with the red meat livestock sector worth A$9.6 billion (ABARE, 2009) being a major component of the economy in both domestic and international trades. The average Australian consumes 46.5 kg of red meat per annum and 10.8 kg of this is sheep meat with 2 kg as mutton (MLA, 2014). This consumption equates to approximately 1.4 serves of lamb meat per week making lamb a significant component of the Australian diet.

The industry value and significant domestic consumption of lamb has led to a need to combat the possible negative health associations of red meat by some consumers. It has been suggested that a diet rich in red meats may increase the risk of cardiovascular disease and colon cancer, which has in turn led to a negative perception of the role of red meat in health and well-being (McAfee *et al.*, 2010a). In the modern western diet, red meat and animal derived foods are a major component of human nutrition. Similarly, in developing countries where affluence is increasing, a trend of increased red meat consumption is occurring (Myers and Kent, 2003). As a result of this increased intake, many nations are concerned for future population health burdens associated with high red meat diets. This could ultimately place pressure on already strained health systems. Therefore increasing the human nutritional health benefits of red meat through genetics and feeding may decrease the risk of chronic disease in diets which are high in red meats (Givens *et al.*, 2006).

Saturated fatty acids (SFA) are considered one of the main health concerns and SFA is relatively high in red meat due to biohydrogenation of diet derived polyunsaturated fatty acids (PUFA) which is exacerbated by acidification of the rumen in grain fed animals (Bauman *et al.*, 2003; Noble, 1981). Consumer education and retail training for “lean lamb” was used to reduce the amount of adipose fat on the average cut of Australian lamb which commenced in the early 1990’s. It is now reported consumers in Australia are eating heavily trimmed, leaner cuts of red meat. This trend no longer makes lamb a major dietary source of SFA (Williams and Droulez, 2010). As a result, consumers are now eating higher levels of intramuscular fat (IMF) which contain different fatty acid profiles and nutrients compared to older high adipose fat containing cuts (Williams and Droulez, 2010).

The omega-3 long-chain (≥C_{20}) polyunsaturated fatty acids (LC omega-3) are essential for human health and wellbeing. The two main LC omega-3 are largely marine-derived and are eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3). These two LC omega-3 have many scientifically proven health benefits for humans (Howe *et al.*, 2006). EPA and DHA are not produced by the human body and need to be ingested. Humans do, however, have the ability to elongate the shorter chain (≤C_{18}) omega-3 alpha linolenic acid (ALA, 18:3ω3) to the LC omega-3 - EPA and DHA - but at an efficiency of approximately 5% in males and 5.5% in females (Burdge, 2004). Therefore, direct intake from dietary sources is considered a more efficient means at achieving the recommended daily intake levels of EPA + DHA.

It has been reported by Howe *et al*. (2006) that the majority of Australians are not consuming the recommended daily intakes of LC omega-3 and that dietary intakes therefore require significant improvement. The majority of LC omega-3 currently consumed by humans is sourced from marine based resources which are under increasing pressure from overfishing, climate change and competing industries (Nichols *et al.*, 2010). Hence, new alternative dietary sources for consumers are of great interest to both industry and consumers (Nichols *et al.*, 2010). Sheep have the ability to convert ALA to LC omega-3 at a similar efficiency to humans (Mortimer *et al.*, 2010) and will deposit LC omega-3 as intramuscular fat therefore providing an alternative, non-marine source (presently at generally low content compared to seafood and other marine sources) of dietary LC omega-3. Pasture reared sheep meat contains dietary “source” (defined as 30 mg/135 g) content of EPA + DHA. These two fatty acids have abundant evidence for positive health benefits for humans. Green grass is rich in LC omega-3 precursor shorter chain (≤C_{18}) omega-3

fatty acid ALA, however, with increased climate variability, it is expected that the availability of green grass will become more limited for prolonged periods and drought-affected sheep have shown significantly lower content of LC omega-3 compared to pasture reared animals.

Previous studies have demonstrated that the intramuscular fat content of LC omega-3 in lamb meat is highly diverse and is affected by a number of factors with diet being identified as a key variable (Wood *et al.*, 2008). The majority of lamb in Australia is reared on pasture and specialist fodder crops which results in lamb containing low but extremely varied, content of intramuscular EPA and DHA (Mortimer *et al.*, 2010; Pannier *et al.*, 2010; Warner *et al.*, 2010). It is evident from prior Australian studies that the availability of green grass is a major factor in determining LC omega-3 content in Australian lamb due to the abundance of the LC omega-3 precursor ALA (Bignell *et al.*, 2011; Mortimer *et al.*, 2010). It has recently been reported that lamb can meet the FSANZ claimable “source” content of 30 mg / 100 g EPA + DHA based on a 135 g serving of lamb entirely reared on irrigated grass at Cowra NSW (Mortimer *et al.*, 2010).

It is well documented that the supplementation of lamb with rumen protected omega-3 or feeding fish oils rich in EPA + DHA will increase IMF EPA + DHA content, but such an approach has not been adopted by industry (Kitessa *et al.*, 2001; Wachira *et al.*, 2002; Noci *et al.*, 2011). Hence finding an alternative method to enhance EPA + DHA content which is readily adoptable by industry is needed and use of an ALA supplement such as canola, linseed or lupin may offer one approach. The cheapest and most abundant source of ALA for grazing livestock is green grass. However, climatic and production limitations results in the majority of Australian lamb typically grazing dry grass from late summer through to early autumn unless augmented with

irrigation and specialised fodders (Duddy *et al.*, 2005). Alongside these typical conditions, the Australian climate is changing and it is predicted extreme seasonal variation will become more frequent with expected increasing periods of drought, prolonged dry periods and higher temperatures occurring in many livestock production regions (PMSEIC, 2007). As a result, the availability of green grass to grazing livestock will be diminished significantly or quality reduced for increased periods of time due to moisture and heat stresses. Therefore drought-affected animals will have to be supplemented more often to meet their daily energy requirements. Given the importance of consumer perception of lamb meat as a healthy and alternative dietary source of LC omega-3, it is pertinent to understand options of supplementation available to maintain the LC omega-3 content of lamb meat during drought periods.

This study is the first attempt at providing a detailed assessment of the impact of drought on LC omega-3 content of Australian lamb raised under drought stress conditions, including with careful examination of the possible influences of breed, sex, genotype, supplementation and environment.

Chapter 2 examines the hypothesis that supplementing animals from a low intake of ALA (drought stressed irrigated pasture) with LA and ALA containing canola meal and lupin meal would improve EPA + DHA content in Australian lambs to dietary "source" levels. Understanding the effects on long-chain omega-3 content of finishing lambs with LA and ALA containing supplements - canola meal and lupin - in a 9 week feeding trial was the aim of this experiment.

Materials and Methods

Animals and experimental design

All animals and procedures utilised in this study had the University of Tasmania Animal Ethics approval (A0009811) and were conducted in accordance with the 1993 Tasmanian Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. A half-sib experimental design was utilised. Five top-EBV rams acquired from Tasmanian Sheep Stud Breeders comprising Dorset, Texel, White Suffolk, East Friesian and Coopworth were mated to purebred Merino ewes at a ratio of 1:120 ewes in separate paddocks in a commercial farming operation in the Coal River Valley, Tasmania, to generate 500 first cross prime lambs.

Animal management

Lambs were marked, vaccinated, electronically tagged at 6 weeks of age and run as one mob per sire group in separate paddocks within the same large scale commercial farming operation under similar management conditions to minimise environmental variation. The flock was raised during a difficult season of severe drought with restricted irrigation capacity. From the third trimester of pregnancy onwards, the animals were raised on a mixture of limited irrigation, drought-affected rye grass pastures without clover and were supplemented with barley. At seven months of age, a representative sub sample of 40 animals with a mean liveweight of 32 ± 2.2 kg and body condition score of 3 were relocated for a 48 day feeding trial.

The animals were individually kept in 0.6 m x 1.2 m metabolic crates in an animal house at Cambridge, Southern Tasmania. Forty sheep comprising of 8 sheep from

each of the 5 sire breeds and assigned to two supplementary feeds (canola or lupin) and two feed levels (1% or 2% of body weight) in which ewes and wethers were equally represented within each sire breed and treatment group. Two animals were removed during the trial for health issues which resulted in a total of 38 samples being collected.

Ration composition

Feed rations were formulated to provide an isocaloric and isonitrogenous basal ration . The rations consisted of 500 g raw barley, 100 g chopped barley straw with a molasses spray at baling, 10 g of multivitamin mineral mix and either 500 g (1%) or 1000 g (2%) of each supplement of canola meal or cracked lupin. All animals had *ad libitum* access to drinking water. Residual feed were recorded and discarded each day and fresh rations mixed for each treatment. Nutrient composition of the experimental and basal rations are depicted on Table 2.1.

Blood sampling

Blood sampling was by jugular venipuncture directly into vacutainers containing EDTA, centrifuged and serum separated from the plasma.

Slaughter and meat sample collection

The prime lambs were slaughtered at Tasmanian Quality Meats at Cressy as per commercial Australian abattoir standards and using the same protocol as JBS Swift kill staff. Carcasses were chilled overnight and freighted to Wurhsthaus Butchery in Cambridge, Southern Tasmanian for full carcass breakdown. *Longissimus dorsi* muscle tissue samples from 38 prime lambs were collected and transported to the laboratory in ice-containing baths and stored at -20°C until ready for genomic DNA and lipid extraction.

Liveweight data

Liveweights were measured monthly using a Ruddweigh 3000XT walk over weighing electronic scale with capability of automatic scanning of lamb identity and downloading of weight data into excel spreadsheets. Daily liveweight gains were then calculated based on final live weight minus initial live weight and divided by number of days in the trial (n=48).

Fatty acid analysis

Longissimus dorsi muscle samples (approximately 1 g) from the 12th rib interface were used for fatty acid analysis. Lipid was extracted using a modified Bligh and Dyer protocol (Bligh and Dyer, 1959). This involved a single phase extraction, $CHCl_3$/MeOH/H_2O (1:2:0.8, by vol.), followed by phase separation to yield a total lipid extract (TLE).

An aliquot of the TLE was trans-methylated in methanol: chloroform; hydrochloric acid (10:1:1, v/v/v) for 2 hours at 80°C. After addition of water, the mixture was extracted three times with hexane:dichloromethane (4:1, v/v, 3x) to obtain fatty acid methyl esters (FAME) which were concentrated under a stream of nitrogen gas.

Samples were made up to a known volume with an internal injection standard (19:0 FAME) added and analysed by gas chromatography (GC) using an Agilent Technologies 7890A GC (Palo Alto, California, USA) equipped with an Supelco Equity-1 fused silica capillary column (15 m×0.1 mm). Helium was used as the carrier gas. Samples were injected, by using a split/splitless injector and an Agilent Technologies 7683B Series auto-sampler operated in splitless mode, at an oven

temperature of 120 °C. After 1 minute, the oven temperature was raised to 270 °C at 10 °C per minute and finally to 300 °C at 5 °C minute which was held for 5 min.

Peaks were quantified by Agilent Technologies GC ChemStation software (Palo Alto, CA, USA). Individual component identification was confirmed by mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC–mass spectrometric analyses were performed on a Finnigan Thermoquest GCQ GC–mass spectrometer fitted with an on-column injector and using Thermoquest Xcalibur software (Austin, TX, USA). The GC was fitted with a capillary column of similar polarity to that described above. GC peak areas were converted to mg/100 g using the 19:0 FAME internal injection standard prior to statistical analysis.

Statistical analyses

Fatty acid data were analysed for the fixed effects of sex, supplement, level of supplementation, sire breed, SNP genotype and their second order interactions using both generalised (PROC GLM) and mixed (PROC MIXED) linear model procedures (SAS 2009), while the partial regressions of sire and herd were fitted as random effects. Least square means of fixed effects were obtained and tested for significance using the Tukey-Kramer adjustment test of paired values.

The full model was

$$Y_{ijklm} = G_i + SG_j + SB_k + SNP_l + (GSG)_{ij} + (GSB)_{ik} + (GSNP)_{il} + (SGSB)_{jk} + (SGSNP)_{jl} + (SBSNP)_{kl} + b_1(S - \overline{S})^2 + b_2(H - \overline{H})^2 + e_{ijklm}$$

where Y_{ijklm} is the $_{ijklm}$th observation of the dependent fatty acid with fixed effects of G_i of ith Gender (i=1,2), SG_j of jth ration size (j=1,2,3), SB_k of kth sire breed

(k=1,2,3,4,5), SNP_l of l^{th} SNP genotype (l=1,2), first order interaction effects $(GSG)_{ij}$, $(GSB)_{ik}$, $(GSNP)_{il}$, $(SGSB)_{jk}$, $(SGSNP)_{jl}$ and $(SBSNP)_{kl}$ of gender and ration size, gender and sire breed, gender and SNP genotype, ration size and sire breed, ration size and SNP genotype and sire breed and SNP genotype respectively. b_1 and b_2 are partial regression coefficients of sire and herd respectively, $b_1(S-\overline{S})^2$ and $b_1(H-\overline{H})^2$ fitted as random effects, and e_{ijklm} is a residual error term normally and independently distributed. All non-significant interactions were later removed from the final model.

Results

The 2% canola (2C) treatment demonstrated the highest average daily gains (ADG) of 112.5 g/day and total liveweight gain of 5.4 kg for the duration of the experiment. In contrast the 2% lupin (2L) treatment had a very low ADG of only 10.1 g/day and animals only gained 1.6 kg (Table 2.2). The 1% treatment groups were similar in growth performance characteristics (Table 2.2).

Fatty acid profiles of the *Longissimus dorsi* muscle tissue are summarised in Tables 2.4 and 2.5 (data expressed as mg/100 g). The overall trends of fatty acid content is broken down into the three major fatty acid groups of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). Sex, breed, level of supplementation and supplement type did not significantly affect the total intramuscular fat (IMF). There was only a slight variation of 0.3% in mean IMF between supplements. Lupin supplemented White Suffolk had the lowest IMF of 2.7±0.6% and lupin supplemented Coopworth and canola supplemented East Friesian had the highest IMF of 4.3 ± 0.6% and 4.3 ± 0.8% respectively.

SFA was the major saturated fatty acid group present with MUFA content also very high (Table 2.6). Palmitic acid (16:0) was the major saturated fatty acid present in all animals. Overall lupin supplemented animals contained 16 mg/100 g more palmitic acid than canola supplemented animals, however, total SFA was almost identical across supplement types (Table 2.6). The minor fatty acid arachidic acid (20:0) was highly significant (P=0.02) for breed with Texel having very high levels present in the canola supplement group. Sex also had a significant (P<0.04) effect on arachidic acid content, with lupin fed males having very low concentrations.

Total MUFA was almost as abundant as SFA (Table 2.6) for both treatments, with a mean MUFA:SFA ratio of 0.97 for the canola and 0.79 for the lupin treatments. However, across breeds there was some variation. Coopworth sired lambs fed canola and their White Suffolk counterparts fed lupins contained more MUFA than SFA overall. The high MUFA:SFA ratio in Coopworth when supplemented with canola (1.06) fell to 0.63 when supplemented with lupin.

MUFA content also increases with supplementation in contrast to drought animals, but like SFA is not significantly affected (P>0.05) by supplement, sex, breed or ration size. Although not statistically significant there is a 163 mg/100g IMF MUFA content difference between canola and lupin treatments (Table 2.7), which may have become significant if the experiment continued. Within the individual MUFA, only one was statistically significant, 18:1ω5c (P=0.02). 18:1ω5c was present at very low content in the IMF and males fed lupin had a lower IMF concentration of this FA.

PUFA content increased with supplementation in grazing animals compared to drought affected animals in the same flock. Total PUFA was very nearly significantly affected (P<0.05) by sex (P=0.052) and the IMF contents of a number of individual LC omega-3 were significantly affected by sex and breed. Total omega-6 PUFA was

significantly affected by breed (P<0.02) with Coopworth having the highest levels (150 mg /100g) than all other breeds and Texel having the lowest (121 mg /100g). The omega-6 PUFA - 18:2ω6 (P=0.04) and 20:3ω6 (P=0.03) - were significantly affected by breed with Texel having significantly lower contents of these two FA compared to other breeds. Sex was significant across a number of PUFA with females demonstrating greater PUFA content and in particular total omega-3 (P<0.05). The fatty acids 20:5ω3, 20:3ω6, 22:6ω3 and 22:5ω3 were all higher in females across the experiment. Ration size or supplement type did not demonstrate a significant (P<0.05) effect on any of the fatty acids. A further PUFA, 20:2ω6, was also present at a very low concentration with a maximum of 1.1 mg/100 g and was statistically significant (P=0.04).

Table 2.1 Dietary composition of feed formulation components.

Nutritional Component	Canola Meal	Cracked Lupin	Barley	Barley straw + molasses
Dry Matter (%)	96.3	93.3	92.0	92.5
Crude Fibre (%)	13.8	15.7	4.6	41.3
Neutral Detergent Fibre (%)	18.9	25.0	14.4	66.4
Acid Detergent Fibre (%)	15.9	20.9	5.5	43.4
Metabolisable Energy (MJ/Kg)	14.9	12.2	13.2	7.3
Digestible Energy (MJ/Kg)	277.3	183.7	213.3	62.3
Feed Digestibility (%)	60.0	40.0	60.0	20.0
Nitrogen (%)	5.3	4.8	1.7	1.0
Crude Protein (%)	33.3	30.1	10.4	6.2
Fat (%)	15.8	6.0	2.3	1.0
Ash (%)	5.9	2.7	2.5	9.6
Shorter chain omega-3 precursor fatty acid ALA (mg/100 g)	517	248	134	Not tested

Table 2.2 Mean performance characteristics of lambs by treatment group n=38 (mean values ± standard errors).

	Treatments			
Variable	**1% Canola**	**2% Canola**	**1% Lupin**	**2% Lupin**
Initial Liveweight	35.1 ± 0.4	35.4 ± 0.3	33.1 ± 0.4	34.3 ± 0.5
Final Liveweight	39.2 ± 0.5	40.8 ± 0.5	37.8 ± 0.6	35.9 ± 0.2
Total Feed Intake (Kg)	39.7 ± 0.8	45.1 ± 0.7	38.5 ± 0.7	39.4 ± 0.6
Daily liveweight gain (g /day)	85.4 ± 5.0	112.5 ± 5.1	97.9 ± 3.3	33.3 ± 10.1

Table 2.3 Statistical significance (p-values) for the effects of sire breed, sex, supplement and level of supplementation on muscle fatty acid profiles

Fatty Acid	Breed	Sex	Supplementation level	Supplement Type
14:0	0.08	0.21	0.72	0.52
15:0	0.55	0.81	0.72	0.93
16:1ω7c	**0.03****	0.06	0.89	0.17
16:1ω7t	0.44	0.09	0.28	0.26
16:0	0.20	0.14	0.72	0.38
17:0	0.10	0.05	0.86	0.54
18:3ω6 GLA	0.71	0.43	0.95	0.76
18:4ω3	0.30	0.07	0.27	0.85
18:2	0.30	0.35	0.12	0.70
18:2ω6 LA	**0.04***	0.16	0.45	0.47
18:3ω3 ALA	0.83	0.31	0.65	0.68
18:1ω9c OA	0.37	0.55	0.73	0.26
18:1ω7c	0.07	0.09	0.09	0.97
18:1ω7t	0.20	0.38	0.19	0.89
18:1ω5c	0.12	**0.04***	0.44	0.46
18:0	0.35	0.34	0.92	0.64
20:4ω6 ARA	0.18	0.17	0.83	0.13
20:5ω3 EPA	0.40	**0.04***	0.83	0.79
20:3ω6	**0.03***	**0.03***	0.94	0.97
20:4ω3	0.31	0.88	0.25	0.90
20:2ω6	0.64	**0.02****	0.33	0.70
20:1ω9#	0.12	0.08	0.09	0.71
20:1ω7c	0.17	0.20	0.56	0.62
20:0	**0.02****	**0.04***	0.30	0.95
22:6ω3 DHA	0.77	**0.05***	0.82	0.49
22:4ω6	0.53	0.43	0.66	0.57
22:5ω3 DPA	0.56	**0.01*****	0.93	0.82
22:0	0.30	0.54	0.74	0.89
23:0	0.88	0.80	0.49	0.81
24:1ω9c	0.87	0.58	0.67	0.30
24:0	0.37	0.73	0.16	0.86
SFA	0.24	0.19	0.86	0.89
MUFA	0.44	0.41	0.84	0.97
PUFA	0.11	0.05	0.60	0.51
EPA + DHA	0.64	**0.02****	0.87	0.34
EPA + DPA + DHA	0.66	**0.02***	0.94	0.57
Total ω3	0.72	**0.05***	0.78	0.92
Total ω6	**0.02****	0.09	0.58	0.90
ω6: ω3 Ratio	0.48	0.20	0.96	0.88
% IMF	0.19	0.43	0.27	0.52

* P<0.05 ** P<0.02 ***P<0.001 # May include 20:1ω11c

Table 2.4 Mean intramuscular fatty acid content (± standard errors) for each sire group and supplement type in mg /100g of the longissimus dorsi.

	Sire Breed and Supplement									
	Coopworth		Dorset		East Friesian		Texel		White Suffolk	
Fatty acid	*Canola (4)*	*Lupin (4)*	*Canola (4)*	*Lupin (4)*	*Canola (4)*	*Lupin (4)*	*Canola (4)*	*Lupin (4)*	*Canola (3)*	*Lupin (3)*
14:0	32.8 ± 11.3	56.7 ± 11.1	34.1 ± 10.3	29.7 ± 5.0	44.4 ± 10.1	36.3 ± 7.3	47.4 ± 2.5	32.2 ± 7.7	35.6 ± 4.9	24.0 ± 6.8
15:0	5.1 ± 1.7	9.4 ± 4.5	8.9 ± 4.0	4.4 ± 1.2	7.4 ± 1.7	2.7 ± 1.0	6.4 ± 0.9	7.4 ± 3.6	7.6 ± 1.5	5.9 ± 3.1
16:1ω9c	4.8 ± 1.5	7.3 ± 1.0	6.3 ± 1.0	4.2 ± 1.2	6.0 ± 1.3	3.6 ± 1.2	4.9 ± 0.5	3.7 ± 1.4	4.9 ± 1.2	4.0 ± 1.0
16:1ω7	25.4 ± 8.6	36.4 ± 6.0	32.1 ± 5.3	27.4 ± 6.7	29.3 ± 5.0	30.3 ± 3.8	23.8 ± 1.8	22.1 ± 3.7	18.7 ± 2.1	20.0 ± 5.0
16:0	381.6 ± 84.7	572.6 ± 91.0	484.5 ± 60.3	416.2 ± 102.7	419.2 ± 72.6	398.8 ± 48.0	404.3 ± 24.8	346.8 ± 50.2	331.0 ± 51.2	372.9 ± 84.9
17:0	46.1 ± 7.2	62.2 ± 4.3	55.4 ± 6.7	48.3 ± 10.4	40.2 ± 7.9	40.4 ± 7.8	47.4 ± 4.8	43.2 ± 9.3	37.9 ± 3.5	44.0 ± 6.8
17:1ω8c	20.0 ± 5.6	30.4 ± 5.0	25.4 ± 4.1	21.2 ± 5.9	22.7 ± 3.9	18.6 ± 2.3	20.0 ± 1.9	18.7 ± 3.3	16.4 ± 3.2	18.2 ± 3.7
18:3ω6 GLA	1.1 ± 0.4	1.2 ± 0.2	0.8 ± 0.3	1.0 ± 0.1	0.7 ± 0.1	1.4 ± 0.2	0.4 ± 0.2	0.9 ± 0.3	0.6 ± 0.1	1.1 ± 0.0
18:4ω3	3.4 ± 0.8	3.1 ± 0.7	3.5 ± 0.5	2.6 ± 0.6	3.3 ± 0.4	4.4 ± 1.2	2.9 ± 0.4	3.3 ± 0.6	2.4 ± 0.2	3.0 ± 0.5
18:2	1.6 ± 0.7	0.5 ± 0.3	4.0 ± 0.5	0.4 ± 0.2	3.4 ± 0.3	0.9 ± 0.9	2.7 ± 0.4	1.2 ± 1.1	1.5 ± 0.2	0.6 ± 0.4
18:2ω6	99.8 ± 12.3	127.8 ± 19.1	115.1 ± 8.9	97.0 ± 19.4	82.4 ± 12.1	121.9 ± 11.4	87.1 ± 7.0	97.7 ± 11.4	73.6 ± 9.5	111.5 ± 13.6
18:3ω3 ALA	15.0 ± 1.9	17.1 ± 2.0	20.8 ± 3.7	13.2 ± 3.5	18.8 ± 4.3	14.0 ± 1.8	17.6 ± 1.0	11.7 ± 2.3	16.3 ± 1.7	11.0 ± 1.8
18:1ω9c OA	708.5 ± 161.6	579.8 ± 148.4	683.1 ± 147.0	464.3 ± 19.0	589.2 ± 183.0	523.0 ± 86.5	536.0 ± 83.3	561.6 ± 79.3	552.1 ± 101.2	684.5 ± 158.6
18:1ω7c	44.3 ± 9.9	37.4 ± 6.6	94.5 ± 7.7	27.4 ± 7.5	65.3 ± 7.5	38.6 ± 10.5	63.6 ± 9.4	32.8 ± 10.3	38.9 ± 1.5	28.6 ± 5.4
18:1ω7t	63.6 ± 17.5	56.3 ± 6.9	152.3 ± 16.7	35.7 ± 6.2	123.2 ± 15.4	42.6 ± 11.3	104.8 ± 19.0	43.2 ± 15.0	61.2 ± 7.5	40.6 ± 11.6
18:1ω5c	6.1 ± 1.5	9.4 ± 0.7	10.6 ± 0.4	4.7 ± 1.2	8.4 ± 1.3	5.8 ± 1.0	8.8 ± 1.1	5.9 ± 1.6	7.9 ± 1.5	4.7 ± 1.2
18:0	370.9 ± 69.5	518.0 ± 83.8	464.3 ± 52.4	364.2 ± 109.3	365.3 ± 70.4	313.4 ± 41.3	376.8 ± 30.8	324.6 ± 54.4	344.8 ± 65.1	343.2 ± 81.9
CLA	8.6 ± 3.3	12.5 ± 1.9	10.7 ± 2.4	8.3 ± 3.7	8.5 ± 1.2	7.7 ± 2.4	9.4 ± 0.9	7.7 ± 2.2	9.2 ± 2.8	9.2 ± 1.9
20:4ω6 ARA	32.7 ± 3.0	31.2 ± 6.7	25.8 ± 4.1	32.0 ± 6.8	16.1 ± 2.5	35.2 ± 2.8	19.0 ± 4.0	29.7 ± 2.8	20.4 ± 2.3	31.0 ± 2.9
20:5ω3 EPA	10.0 ± 0.5	10.0 ± 3.1	11.4 ± 3.3	10.7 ± 2.2	7.5 ± 1.5	12.2 ± 1.8	8.9 ± 2.7	8.8 ± 1.4	7.5 ± 1.0	8.6 ± 2.1
20:3ω6	4.2 ± 0.4	4.6 ± 0.9	4.0 ± 0.4	4.3 ± 0.7	2.6 ± 0.4	5.1 ± 0.5	3.5 ± 1.1	3.9 ± 0.3	2.8 ± 0.4	4.8 ± 0.4
20:1	1.3 ± 0.6	0.9 ± 0.4	2.2 ± 1.2	0.8 ± 0.4	1.5 ± 1.0	1.4 ± 0.5	2.5 ± 1.9	0.5 ± 0.3	0.7 ± 0.2	1.3 ± 0.2
20:1ω9	3.5 ± 0.6	3.0 ± 0.5	5.9 ± 0.3	1.9 ± 0.6	4.5 ± 0.5	3.0 ± 1.1	4.6 ± 0.6	2.0 ± 0.9	2.8 ± 0.4	2.2 ± 0.6
20:1ω7c	0.6 ± 0.3	0.5 ± 0.2	0.9 ± 0.3	0.0 ± 0.0	0.5 ± 0.1	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.2	0.0 ± 0.0	0.2 ± 0.2
20:0	3.9 ± 0.9	4.6 ± 0.7	5.3 ± 0.3	3.2 ± 1.0	4.2 ± 0.7	3.2 ± 0.6	6.5 ± 0.8	3.5 ± 1.0	3.6 ± 0.5	2.8 ± 0.6
22:6ω3 DHA	3.9 ± 0.5	3.3 ± 1.1	3.5 ± 0.8	3.4 ± 0.7	3.5 ± 1.1	6.9 ± 2.0	2.8 ± 0.7	3.1 ± 0.6	3.3 ± 0.2	3.9 ± 0.9
22:4ω6	1.4 ± 0.2	1.3 ± 0.1	0.9 ± 0.3	1.3 ± 0.3	0.4 ± 0.2	1.5 ± 0.1	0.5 ± 0.3	1.3 ± 0.1	0.6 ± 0.3	1.6 ± 0.1
22:5ω3 DPA	10.4 ± 0.7	9.5 ± 2.5	9.7 ± 2.2	10.0 ± 1.6	7.0 ± 1.3	10.8 ± 1.3	8.7 ± 1.7	9.1 ± 0.9	8.3 ± 0.8	9.0 ± 1.3
22:0	1.9 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.3	1.4 ± 0.4	2.1 ± 0.1	2.3 ± 0.6	1.5 ± 0.5	1.4 ± 0.1	1.8 ± 0.1
24:0	1.7 ± 0.1	1.7 ± 0.2	1.6 ± 0.2	1.4 ± 0.3	1.4 ± 0.4	1.3 ± 0.5	1.3 ± 0.2	1.3 ± 0.4	1.3 ± 0.1	1.7 ± 0.1
EPA+DHA	13.9 ± 0.9	13.3 ± 4.2	14.9 ± 4.0	14.1 ± 2.9	10.9 ± 2.6	19.1 ± 3.6	11.7 ± 3.3	11.9 ± 2.0	10.9 ± 1.2	12.5 ± 2.9
EPA+DPA+DHA	24.3 ± 1.6	22.8 ± 6.6	24.6 ± 6.2	24.1 ± 4.4	17.9 ± 3.8	29.9 ± 4.9	20.3 ± 4.9	21.0 ± 2.9	19.1 ± 1.9	21.5 ± 4.2
Total SFA	845.1 ± 171.4	1228.2 ± 190.9	1056.7 ± 125.5	870.0 ± 228.4	884.0 ± 160.0	799.0 ± 99.3	893.3 ± 61.3	761.0 ± 123.8	764.1 ± 119.7	797.1 ± 184.1
Total MUFA	895.4 ± 195.8	774.4 ± 150.7	1036.9 ± 171.5	596.8 ± 45.0	873.0 ± 220.4	677.7 ± 77.5	789.6 ± 108.7	700.0 ± 115.4	719.2 ± 113.4	813.6 ± 185.7
Total PUFA	208.0 ± 20.8	233.6 ± 24.0	231.9 ± 26.1	192.4 ± 37.9	174.8 ± 27.8	232.0 ± 24.8	182.9 ± 16.0	187.3 ± 23.2	161.1 ± 13.8	204.1 ± 21.4
Total Omega 3	42.9 ± 3.4	43.1 ± 7.7	49.1 ± 9.8	40.0 ± 7.6	40.8 ± 8.3	49.5 ± 7.9	41.3 ± 6.0	36.9 ± 5.2	37.8 ± 3.4	35.7 ± 5.7
Total Omega 6	139.1 ± 14.3	166.0 ± 20.7	146.5 ± 12.6	135.7 ± 25.8	102.0 ± 14.8	165.2 ± 14.5	110.6 ± 12.2	133.5 ± 14.5	98.0 ± 10.4	150.1 ± 13.6
ω6 : ω3 Ratio	3.2 ± 0.1	4.2 ± 0.9	3.2 ± 0.4	3.4 ± 0.1	2.6 ± 0.3	3.5 ± 0.3	2.7 ± 0.2	3.7 ± 0.3	2.6 ± 0.1	4.3 ± 0.5
Total IMF %	3.1 ± 0.6	4.3 ± 0.6	3.9 ± 0.5	3.1 ± 0.3	4.3 ± 0.8	3.4 ± 0.8	3.8 ± 0.4	3.1 ± 0.2	3.5 ± 1.0	2.7 ± 0.6

Table 2.5 Mean intramuscular fatty acid content (±standard errors) for sex and supplement type in mg / 100g of the longissimus dorsi.

	Sex and Supplement			
	Canola		Lupin	
Fatty acid	*Male (n=9)*	*Female (n=10)*	*Male (n=10)*	*Female (n=9)*
14:0	39.9 ± 5.5	38.3 ± 5.5	31.1 ± 5.6	41.2 ± 5.8
15:0	8.5 ± 1.7	5.7 ± 0.9	3.9 ± 1.7	7.8 ± 1.9
16:1ω9c	5.7 ± 0.6	5.1 ± 0.8	3.8 ± 0.9	5.4 ± 0.7
16:1ω7	26.0 ± 2.2	26.5 ± 4.2	23.4 ± 2.6	31.4 ± 3.8
16:0	422.6 ± 30.3	394.8 ± 46.0	361.2 ± 39.7	480.6 ± 54.6
17:0	45.1 ± 3.5	46.4 ± 4.8	39.8 ± 5.2	55.0 ± 4.3
17:1ω8c	21.5 ± 2.1	20.8 ± 2.8	18.9 ± 2.4	24.0 ± 3.1
18:3ω6 GLA	0.6 ± 0.1	0.8 ± 0.2	1.1 ± 0.2	1.1 ± 0.1
18:4ω3	2.9 ± 0.3	3.3 ± 0.4	3.0 ± 0.6	3.6 ± 0.4
18:2	3.0 ± 0.3	2.4 ± 0.5	0.3 ± 0.1	1.1 ± 0.5
18:2ω6	89.1 ± 7.3	95.6 ± 7.8	102.4 ± 10.5	119.1 ± 8.8
18:3ω3 ALA	17.8 ± 1.7	17.7 ± 1.9	11.1 ± 1.1	15.7 ± 1.5
18:1ω9c OA	629.0 ± 71.1	606.3 ± 96.9	518.3 ± 65.6	590.3 ± 61.5
18:1ω7c	68.4 ± 7.5	57.1 ± 8.3	25.2 ± 2.4	40.3 ± 5.7
18:1ω7t	114.7 ± 11.9	92.7 ± 16.7	35.0 ± 5.2	51.8 ± 6.4
18:1ω5c	8.8 ± 0.6	8.0 ± 1.0	5.1 ± 0.7	7.1 ± 0.9
18:0	418.8 ± 34.4	357.4 ± 36.0	306.1 ± 41.0	435.6 ± 51.0
CLA	9.2 ± 0.9	9.3 ± 1.6	6.7 ± 1.1	11.2 ± 1.6
20:4ω6 ARA	19.8 ± 2.2	25.8 ± 2.9	30.8 ± 2.6	32.8 ± 3.1
20:5ω3 EPA	7.5 ± 0.9	10.6 ± 1.4	9.1 ± 1.2	11.0 ± 1.4
20:3ω6	3.0 ± 0.3	3.9 ± 0.4	4.1 ± 0.3	4.9 ± 0.4
20:2ω6	0.9 ± 0.3	2.4 ± 0.9	0.8 ± 0.2	1.1 ± 0.3
20:1ω9*	4.8 ± 0.4	3.9 ± 0.5	1.7 ± 0.4	3.1 ± 0.4
20:1ω7c	0.6 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.1
20:0	5.0 ± 0.5	4.5 ± 0.6	2.8 ± 0.4	4.1 ± 0.5
22:6ω3 DHA	3.1 ± 0.5	3.6 ± 0.3	3.4 ± 0.5	4.8 ± 1.0
22:4ω6	0.6 ± 0.2	0.9 ± 0.2	1.3 ± 0.0	1.5 ± 0.1
22:5ω3 DPA	7.6 ± 0.6	10.0 ± 1.0	8.7 ± 0.7	10.6 ± 1.1
22:0	1.6 ± 0.1	1.8 ± 0.3	1.5 ± 0.2	2.0 ± 0.1
24:0	1.5 ± 0.2	1.5 ± 0.1	1.3 ± 0.2	1.7 ± 0.2
EPA+DHA	10.7 ± 1.4	14.2 ± 1.7	12.5 ± 1.7	15.8 ± 2.2
EPA+DPA+DHA	18.2 ± 2.0	24.2 ± 2.7	21.3 ± 2.3	26.4 ± 3.2
Total SFA	944.0 ± 70.3	851.3 ± 91.3	748.4 ± 90.0	1028.8 ± 115.3
Total MUFA	903.7 ± 84.5	840.4 ± 119.7	640.4 ± 76.9	767.3 ± 61.3
Total PUFA	186.0 ± 14.0	200.0 ± 16.2	189.8 ± 14.4	228.5 ± 17.1
Total Omega 3	39.1 ± 3.7	45.7 ± 4.3	36.1 ± 3.6	46.0 ± 4.4
Total Omega 6	113.1 ± 9.2	126.9 ± 10.4	139.8 ± 11.3	159.4 ± 11.6
ω6 : ω3 Ratio	3.0 ± 0.2	2.8 ± 0.2	4.1 ± 0.4	3.6 ± 0.2
Total IMF %	3.8 ± 0.3	3.7 ± 0.5	3.5 ± 0.5	3.2 ± 0.2

* May include 20:1ω11c

Table 2.6 Mean intramuscular fatty acid content (± standard errors) for supplement type as mg /100g of the longissimus dorsi.

Fatty acid	Supplement	
	Canola(n=19)	*Lupin (n=19)*
14:0	39.0 ± 3.8	36.4 ± 4.1
15:0	7.1 ± 1.0	6.0 ± 1.3
16:1ω9c	5.4 ± 0.5	4.6 ± 0.6
16:1ω7	26.2 ± 2.4	27.6 ± 2.5
16:0	408.0 ± 27.6	424.0 ± 36.3
17:0	45.8 ± 2.9	47.8 ± 3.7
17:1ω8c	21.1 ± 1.7	21.6 ± 2.0
18:3ω6 GLA	0.7 ± 0.1	1.1 ± 0.1
18:4ω3	3.1 ± 0.2	3.3 ± 0.4
18:2	2.7 ± 0.3	0.7 ± 0.3
18:2ω6	92.5 ± 5.3	111.2 ± 6.9
18:3ω3 ALA	17.8 ± 1.3	13.5 ± 1.1
18:1ω9c OA	617.0 ± 59.5	556.2 ± 44.4
18:1ω7c	62.5 ± 5.6	33.2 ± 3.6
18:1ω7t	103.1 ± 10.5	43.8 ± 4.5
18:1ω5c	8.4 ± 0.6	6.2 ± 0.6
18:0	386.5 ± 25.3	374.2 ± 35.6
CLA	9.3 ± 0.9	9.1 ± 1.1
20:4ω6 ARA	22.9 ± 1.9	31.9 ± 2.0
20:5ω3 EPA	9.1 ± 0.9	10.1 ± 0.9
20:3ω6	3.5 ± 0.3	4.5 ± 0.3
20:2ω6	1.7 ± 0.5	0.9 ± 0.2
20:1ω9*	4.4 ± 0.3	2.4 ± 0.3
20:1ω7c	0.5 ± 0.1	0.3 ± 0.1
20:0	4.7 ± 0.4	3.5 ± 0.3
22:6ω3 DHA	3.4 ± 0.3	4.1 ± 0.6
22:4ω6	0.7 ± 0.1	1.4 ± 0.1
22:5ω3 DPA	8.8 ± 0.7	9.7 ± 0.7
22:0	1.7 ± 0.2	1.8 ± 0.1
24:0	1.5 ± 0.1	1.5 ± 0.1
EPA+DHA	12.5 ± 1.2	14.3 ± 1.4
EPA+DPA+DHA	21.4 ± 1.8	24.0 ± 2.1
Total SFA	895.2 ± 57.9	896.0 ± 79.3
Total MUFA	870.4 ± 73.0	707.2 ± 49.6
Total PUFA	193.3 ± 10.6	210.2 ± 11.9
Total Omega 3	42.6 ± 2.9	41.3 ± 3.0
Total Omega 6	120.4 ± 7.0	150.1 ± 8.2
ω6 : ω3 Ratio	2.9 ± 0.1	3.8 ± 0.2
Total IMF %	3.7 ± 0.3	3.4 ± 0.2

*May contain 20:1ω11c

Discussion

The ability of ALA rich cracked lupin and canola meal to remediate the negative effects of suppressed intake of ALA rich green grass due to drought is possible as evident in Figure 2.1. A large increase in intramuscular LC omega-3 content occurred in supplemented animals, but intramuscular content still did not meet the FSANZ claimable “source” of 30 mg/100 g. The mean intramuscular EPA + DHA content nearly doubled in comparison to drought affected animals (7.5 mg/100 g) and after 48 days of supplementation EPA + DHA content increased to 12.5 mg/100 g with canola and 14.3 mg/100 g with cracked lupin (Table 2.7).

This finding is similar to a number of recent studies which demonstrate that feeds with high levels of ALA can lead to higher content of intramuscular LC omega-3 compared to feeds with low ALA (Aurousseau *et al.*, 2007a; Demise *et al.*, 1998; Mortimer *et al.*, 2010). These studies contrasted pasture-reared animals with a number of different supplements and conserved forage such as hay and silage. The current experiment differed from the aforementioned studies in the manner that it used breeds which were not adapted to semi-arid conditions (drought) and were then introduced to supplements 48 days prior to slaughter to attempt recovery of decreased intramuscular LC omega-3 content and growth rates due to limited green grass intake.

Growth Rates

The animals did not appear to develop a period of compensatory growth after removing from drought affected pastures and introduced to confined feeding and

supplementation; all treatment groups demonstrated reasonable average daily gains post 21 day rumen adaptation. The very low liveweight gain of the 2% lupin group suggest that a high level of supplementation suppresses digestibility in the rumen, hence, most of the ingested nutrients were not efficiently utilised for weight gain, but passed out of the gastro-intestinal tract as faecal droppings.

Average daily gains are much lower compared to modern industry growth rates in excess of 300 g/day (Jolly and Wallace, 2007). The majority of animals with high ADG reported in Jolly and Wallace (2007) were from animals of higher liveweight at commencement of feeding and for significantly longer durations. Growth rates comparable to our observed rates have been reported in first cross Merinos at similar age, however, entry liveweight still appears low indicating that this supplementation level was not promoting optimal liveweight gain (Kirby and Beretta, 2004; Suiter and McDonald, 1987). Initial liveweight may not be a significant factor in decreased ADG as a recent UK study of crossbred hill sheep, which included Texel's, achieved ADG in excess of 200 g liveweight per day with rations which included canola seed (Annett *et al.*, 2011). The lower growth rates observed during this feeding trial were mostly attributable to the high dietary energy density of the supplementary feeds. It is widely recognised that the higher the energy density of a given feed, the lower the dry matter intake of animals. This negative correlation between feed intake and dietary energy content impacts on satiety levels and indirectly, on growth rates of animals.

Saturated and Monounsaturated Fatty Acid Profiles

The SFA contents did increase with the introduction of supplements compared with drought affected animals from the same flock slaughtered at the commencement of the feeding trial. The initial SFA content values in the drought flock are similar to

values obtained in a study using the semi-arid breed Barbarine (Atti and Mahouachi, 2009). SFA content increased as a result of confined supplement feeding, however, supplement type was not a significant factor ($P<0.05$).

Canola does contain more than double the concentration of arachidic acid, yet supplement type was not significant (Table 2.3). Arachidic acid can be produced by the hydrogenation of arachidonic acid (ARA, 20:4ω6) and perhaps Texel males fed lupins had lower levels of biohydrogenation of ARA to AA. Both supplement types had negligible IMF AA content.

A number of studies of fatty acids in lambs fed concentrates versus pasture have demonstrated an increase in SFA (Atti and Mahouachi, 2009; Aurousseau *et al.*, 2004; Aurousseau *et al.*, 2007a; Mortimer *et al.*, 2010) which ultimately does decrease the health benefits in comparison to green pasture-reared animals. However, trimmed red meat contributes less than 10% of dietary SFA in Australia and is not considered a major source of SFA, so this minor increase due to supplementation is likely not as negative on human health as initially perceived (Williams and Droulez, 2010).

PUFA Content

Linoleic acid (LA, 18:2ω6) is the precursor FA in the ω6 pathway with 20:3ω6 (Dihomo-γ-linolenic acid, DGLA) also being an intermediate FA in the pathway (Qi et al., 2004). Studies into the effect of sex upon EPA + DHA content have shown human females are slightly more efficient than men in storing EPA + DHA (Burdge, 2004), however, this trend has not been observed in Australian lambs (Mortimer et

al., 2010). In contrast to Kitessa et al. (2010), sex has shown a significant (P=0.02) effect on LC omega-3 content when supplementing drought affected lamb with canola and lupin. Both canola and lupin are rich sources of ALA with canola containing nearly twice as much ALA than lupin. Despite nearly double the ALA concentration in canola, both sexes had similar IMF ALA content which indicates ration structures were too high in supplements to create FA segregation of ALA due to supplement type. The significant (P= 0.01) association for sex of the EPA to DHA intermediate DPA and significant associations for EPA (P=0.04) and DHA (P=0.05) suggests that when drought stressed animals are supplemented, ewes will deposit LC omega-3 at a higher rate compared to males.

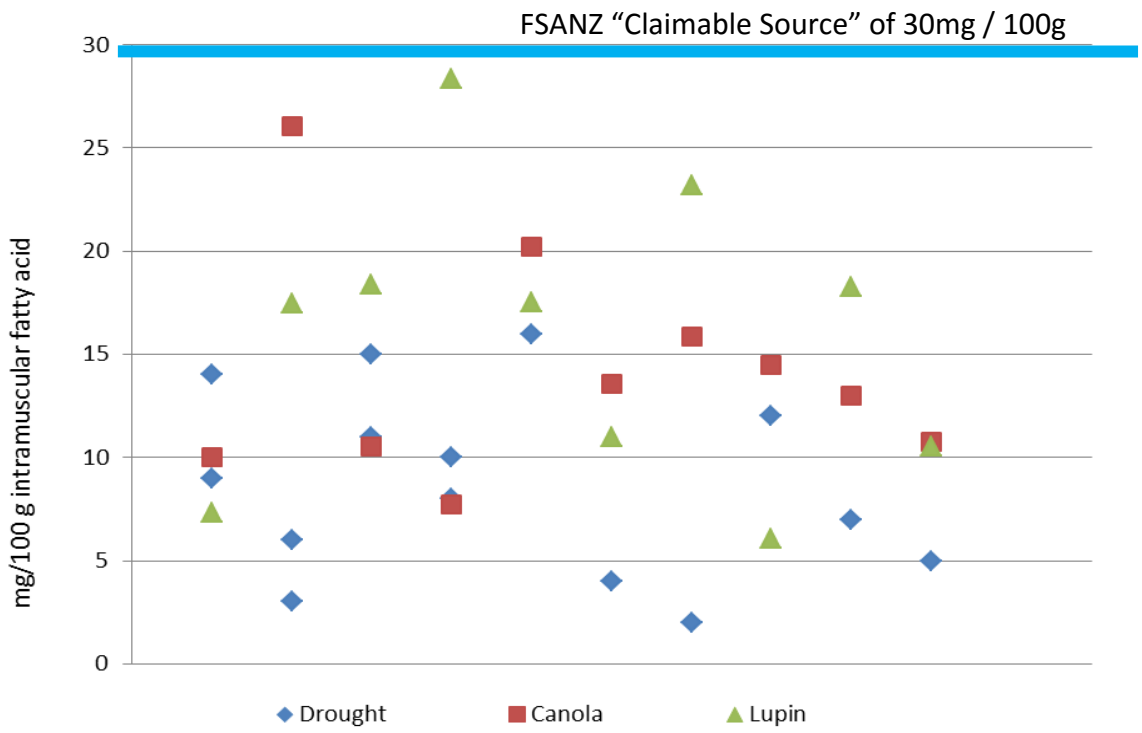

Figure 2.1 Scatter plot of individual EPA + DHA intramuscular content (mg/100 g) for pasture, canola and lupin fed animals distributed along the x axis to reduce clutter and highlight extreme variations. 96 fast growing lambs reared on drought affected pasture (Drought) were slaughtered at the commencement of the feeding trial and are assumed to be representative of the typical content of intramuscular EPA + DHA at commencement of the trial. The canola and

lupin data points indicate the content of EPA + DHA at the conclusion of 60 days supplementation.

There was a reasonable degree of variation in IMF omega-3 content, but not as high as was expected. Recent studies into the IMF content of LC omega-3 EPA and DHA in lamb have demonstrated that there is as much variation between individuals as there is between breeds, sex and treatments (Mortimer *et al.*, 2010; Wijesundera *et al.*, 2011; Aurousseau *et al.*, 2007a; Dervishi *et al.*, 2012). Reported changes in the IMF omega-3 content by supplementation vary significantly according to supplement type and duration. Most recently, Kitessa *et al.* (2010) assessed lambs that initially grazed Kikuyu pastures and then later moved to a confined feeding system utilizing a commercial concentrate pellet or concentrate plus linseed. The concentrate plus linseed increased mean EPA + DHA content by 11.5 mg/ 100 g compared to pure concentrate alone which allowed the meat to reach FSANZ dietary “source” levels of 30 mg/100g based upon a serve of 140 g raw meat.

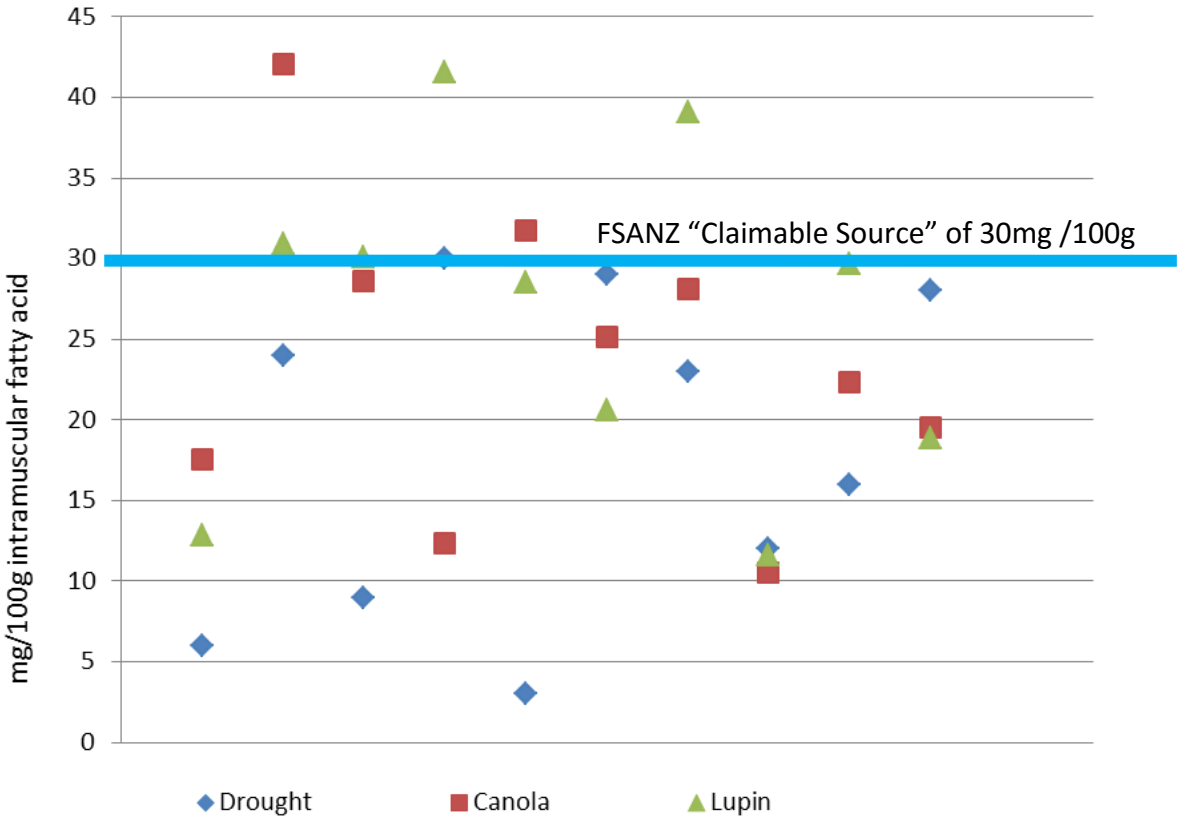

Figure 2.2 Scatter plot of individual EPA + DHA + DPA intramuscular content for n=96 drought-affected pasture, canola and lupin fed animals distributed along the x axis to reduce clustering. Intramuscular DPA is present as the main LC omega-3 when included in the LC omega-3 calculations. Inclusion of DPA effectively doubles the intramuscular LC omega-3 content with a number of animals exceeding 30mg / 100g. However DPA is not a claimable FSANZ LC omega-3 at present.

The results of this feeding trial showed that the negative effects of low green grass intake over summer can be remediated with the use of ALA rich supplements. However, in the present trial using canola meal and cracked lupins, it was demonstrated that animals which were reared entirely under severe drought conditions and then later supplemented could not raise the content of EPA + DHA to meet "source" claims even based upon a 135 g raw serve.

Conclusion

Canola meal and cracked lupins have a positive effect on the decreased long-chain omega-3 content in severe drought-raised Australian lamb. Despite this remedial effect, supplementation still does not bring the claimable EPA + DHA content up to FSANZ claimable dietary “source” levels of 30 mg/100 g. This finding shows that despite supplementation with ALA rich canola meal or cracked lupin, drought has a negative effect on the healthy eating characteristics of Australian lamb. In contrast to other lamb studies, this trial demonstrated a clear sex effect where females deposited more intramuscular EPA + DHA and DPA than males. Future research into LC omega-3 enhancement requires techniques which are readily adoptable into the Australian sheep meat industry. This may include the potential use of molecular marker breeding tools for targeted breeding, via selection of breeding stock containing elevated LC omega-3 content, which can be coupled with use of ALA rich supplements. Such research is required to maintain consumer preference and industry claims for healthy lamb meat to consistently meet the FSANZ “source” levels of 30 mg/100 g including during prolonged drought.

Supplementary Tables and Figures

Table 2.7 Summary of significance values for meat quality against dependent variables which were tested in the analysis of the data in this chapter but not included in the results of this chapter.

	Breed	Sex	Ration	FADS2	FABP4_1	FABP4_2
Hot Carcass Weight	0.82	0.84	0.52	0.13	0.80	0.84
Carcass Yield	0.38	0.94	0.64	0.12	0.91	0.58
Fat Score	0.12	0.26	0.32	0.23	0.21	0.25
Subcutaneous Fat	0.99	0.06	0.15	0.56	0.60	0.52
Longissimus dorsi Length	0.68	0.10	0.35	0.60	0.07	0.46
Longissimus dorsi Width	0.22	0.78	0.74	0.22	0.74	0.77
Longissimus dorsi Area cm2	0.72	0.94	0.65	0.88	0.84	0.35
Longissimus dorsi Calculation	0.11	0.56	0.37	0.09	0.38	0.57
Short Loin Muscle Weight	0.34	0.80	0.93	0.11	0.89	0.47
Short loin Bone weight	0.05*	0.98	0.64	0.71	0.32	0.60
Short Loin Total Weight	0.25	0.93	0.71	0.49	0.46	0.94
Tender Loin	0.21	0.27	0.68	0.46	0.30	0.33
Trim Weight	0.41	0.24	0.84	0.57	0.69	0.60
Forequarter Boned Left	0.70	0.69	0.23	0.09	0.76	0.82
Forequarter Boned Right	0.92	0.64	0.58	0.20	0.81	0.84
Shanks	0.34	0.15	0.48	0.32	0.71	0.40
Leg Roast	0.35	0.18	0.08	0.44	0.09	0.14
Boned Leg Roast	0.96	0.83	0.75	0.33	0.94	0.12
Forequarter Trim	0.05*	0.28	0.97	0.31	0.26	0.09
Boned racks	0.79	0.08	0.28	0.76	0.51	0.22
Total Cuts Weight	0.42	0.83	0.59	0.13	0.69	0.86

References

ABARE (2009). The value of the red meat industry to Australia. In *ABARE Research report*(Eds S. Fletcher, B. Buetre and K. Morey). Australian Government -

Annett, R. W., Carson, A. F., Dawson, L. E. R. & Kilpatrick, D. J. (2011). Effects of dam breed and dietary source of n-3 polyunsaturated fatty acids on the growth and carcass characteristics of lambs sourced from hill sheep flocks. *Animal* 5(7): 1023-1035.

Atti, N. & Mahouachi, M. (2009). Effects of feeding system and nitrogen source on lamb growth, meat characteristics and fatty acid composition. *Meat Science* 81(2): 344-348.

Aurousseau, B., Bauchart, D., Calichon, E., Micol, D. & Priolo, A. (2004). Effect of grass or concentrate feeding systems and rate of growth on triglyceride and phospholipid and their fatty acids in the M-longissimus thoracis of lambs. *Meat Science* 66(3): 531-541.

Aurousseau, B., Bauchart, D., Faure, X., Galot, A. L., Prache, S., Micol, D. & Priolo, A. (2007). Indoor fattening of lambs raised on pasture. Part 1: Influence of stall finishing duration on lipid classes and fatty acids in the longissimus thoracis muscle. *Meat Science* 76(2): 241-252.

Bauman, D. E., Perfield II, J. W., de Veth, M. J. &Lock, A. L. (2003).New perspectives on lipid digestion and metabolism in ruminants. In *Proc. Cornell Nutr. Conf.*, 175-189.

Bignell, C. W., Nichols, P. D., Malau-Aduli, A. E. O., Kijas, J. W. & McCulloch (2011).Intramuscular long-chain omega 3 content in Australian lamb: Drought effect, genomics and dieatary improvment stratergies. In *Australasian Section of the American Oil Chemists Society*Adelaide.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37(8): 911-917.

Burdge, G. (2004). Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. *Current Opinion in Clinical Nutrition and Metabolic Care* 7(2): 137-144.

Demise, S., Matthes, H. D., Mohring, H., Nurnberg, K., Bittner, G., Pilz, K., Hartung, M. &Schubert, M. (1998). Investigations on the influence of breed, sex and feeding on lamb carcass composition and quality. *Zuchtungskunde* 70(2): 119-140.

Dervishi, E., Joy, M., Alvarez-Rodriguez, J., Serrano, M. & Calvo, J. H. (2012). The forage type (grazing versus hay pasture) fed to ewes and the lamb sex affect fatty acid profile and lipogenic gene expression in the longissimus muscle of suckling lambs1. *Journal of Animal Science* 90(1): 54-66.

Duddy, G., Hegarty, R. S. & White, A. (2005). Managing for lamb production. In *Sheep Production*, 9-1 - 9-20 (Ed D. Cottle). Armidale, Australia: School of Rural Science and Agriculture University of New England.

Givens, D. I., Khem, K. E. & Gibbs, R. A. (2006). The role of meat as a source of n-3 polyunsaturated fatty acids in the human diet. *Meat Science* 74(1): 209-218.

Howe, P., Meyer, B., Record, S. & Baghurst, K. (2006). Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition* 22(1): 47-53.

Jolly, S. & Wallace, A. (2007). Best Practice for production feeding of lambs: a review of the litterature. 179 (Ed B. Brice). Sydney: Meat and Livestock Australia.

Kirby, R. & Beretta, V. (2004). Feeding grain to confined sheep. In *Feeding Grain for Sheep Meat Production*, 33-39 (Ed H. Chapman). Armidale: Australian Sheep Industry CRC.

Kitessa, S. M., Gulati, S. K., Ashes, J. R., Scott, T. W. & Fleck, E. (2001). Effect of feeding tuna oil supplement protected against hydrogenation in the rumen on growth and n-3 fatty acid content of lamb fat and muscle. *Australian Journal of Agricultural Research* 52(4): 433-437.

McAfee, A., McSorley, E., Cuskelly, G., Moss, B., Wallace, J., Bonham, M. & Fearon, A. (2010). Red meat consumption: an overview of the risks and benefits. Vol. 84, 1-1-13 England.

MLA (2010). Australian Sheep Meat Industry Overview. In *Australian Sheep Meat Industry Projections 2010*: Meat and Livestock Australia.

Mortimer, S. I., van der Werf, J. H. J., Jacob, R. H., Pethick, D. W., Pearce, K. L., Warner, R. D., Geesink, G. H., Edwards, J. E. H., Gardner, G. E., Ponnampalam, E. N., Kitessa, S. M., Ball, A. J. & Hopkins, D. L. (2010). Preliminary estimates of genetic parameters for carcass and meat quality traits in Australian sheep. *Animal Production Science* 50(11-12): 1135-1144.

Myers, N. & Kent, J. (2003). New Consumers: The Influence of Affluence on the Environment. *Proceedings of the National Academy of Sciences of the United States of America* 100(8): 4963-4968.

Nichols, P. D., Petrie, J. & Surinder, S. (2010). Long-chain omega-3 oils - an update on sustainable sources. *Nutrients* 2(6): 572-585.

Noble, R. (1981). Digestion, absorption and transport of lipids in ruminant animals. In *Lipid Metabolism in Ruminant Animals*, 57-93 (Ed W. Christie). Oxford, UK: Pergamon Press.

Noci, F., Monahan, F. J. &Moloney, A. P. (2011). The fatty acid profile of muscle and adipose tissue of lambs fed camelina or linseed as oil or seeds. *Animal* 5(1): 134-147.

Pannier, L., Ponnampalam, E. N., Gardner, G. E., Hopkins, D. L., Ball, A. J., Jacob, R. H., Pearce, K. L. &Pethick, D. W. (2010). Prime Australian lamb supplies key nutrients for human health. *Animal Production Science* 50(11-12): 1115-1122.

PMSEIC (2007). Climate Change in Australia: Regional Impacts and Adaptation – Managing the Risk for Australia,. 63 (Eds E. a. I. Prime Minister's Science and Council). Canberra: Commonweath of Australia.

Qi, B., Fraser, T., Mugford, S., Dobson, G., Sayanova, O., Butler, J., Napier, J. A., Stobart, A. K. & Lazarus, C. M. (2004). Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. *Nat Biotech* 22(6): 739-745.

Suiter, R. & McDonald, C. (1987). Growth of Merino weaners fed grain-based diets while grazing dry pasture or housed in feedlots. *Australian Journal of Experimental Agriculture* 27(5): 629-632.

Wachira, A. M., Sinclair, L. A., Wilkinson, R. G., Enser, M., Wood, J. D. & Fisher, A. V. (2002). Effects of dietary fat source and breed on the carcass composition, n-3 polyunsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. *British Journal of Nutrition* 88(6): 697-709.

Warner, R. D., Jacob, R. H., Edwards, J. E. H., McDonagh, M., Pearce, K., Geesink, G., Kearney, G., Allingham, P., Hopkins, D. L. & Pethick, D. W. (2010). Quality of lamb meat from the Information Nucleus Flock. *Animal Production Science* 50(11-12): 1123-1134.

Wijesundera, C., Kitessa, S., Abeywardena, M., Bignell, W. & Nichols, P. D. (2011). Long-chain omega-3 oils: Current and future supplies, food and feed applications, and stability. *Lipid Technology* 23(3): 55-58.

Williams, P. &Droulez, V. (2010). Australian red meat consumption - implications of changes over 20 years on nutrient composition. *Food Australia* 62(3): 87-94.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I. &Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science* 78(4): 343-358.

Chapter 3

Single Nucleotide Polymorphism, supplementary diet and sire breed effects on meat quality traits in first-cross Merino prime lambs.

Abstract

The long term health benefits of consuming long-chain (≥C_{20}) omega-3 polyunsaturated fatty acids (LC omega-3) are becoming widely understood amongst the Australian population. This understanding has led to increased demand for foods rich in omega-3 and in particular the LC omega-3 - eicosapentaenoic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6ω3). It is well documented that animals reared on green grass contain long-chain omega-3 within their intramuscular fats at varying levels. The Australian sheep meat sector is based on pasture and fodder crops and the potential to increase LC omega-3 content and capitalise on consumer demand for omega-3 has great merit for the sheep meat industry.

This study investigated the effects of single nucleotide polymorphic (SNP) loci, sire breed, dietary supplementation with omega-3 polyunsaturated fatty acids and relocation to non-drought affected pastures on the levels of muscle LC omega-3 and meat quality in first cross Merino prime lambs. The F1 lambs (n=354) sired by Texel, East Friesian, Dorset, Coopworth and White Suffolk rams were initially raised on irrigated improved pastures in Southern Tasmania after weaning before relocation to northern Tasmania which was not in drought. Animals were slaughtered in three batches upon attaining the minimum liveweight threshold of 45 kg. The fourth batch of lambs (n=38) was slaughtered at the conclusion of a nine week supplementary feeding trial with lupin and canola meals. Eye muscle area (EMA) and height (EMH)

of the *Longissimus dorsi* between the 12th and 13th ribs were significantly (P<0.001) smaller by 1.9 cm^2 and 27.7mm respectively, in East Friesian sired lambs compared to other breeds. Lambs sired by Dorset and White Suffolk breeds had significantly (P<0.001) more subcutaneous fat than other breeds (25.3 mm). East Friesians were the leanest (20.3 mm) and had the heaviest muscle weight. Animals fed lupin had a shorter eye muscle width, but this did not significantly affect muscle yield. Age at slaughter (slaughter batch) significantly (P=0.0007) affected fat scores and eye muscle shape (P=0.27 for width) and eye muscle area (P=0.0002) where younger animals with a preference for rapid growth had leaner carcasses and smaller eye muscle than older animals at the same carcass weight. Neither FADS2 nor FABP4 SNPs had any significant (P>0.05) effect on any of the meat quality parameters tested. The results indicate that, as long as animals reach the required target liveweight and fat score before slaughter, rearing lambs on drought affected pasture, relocation or supplementation with canola or lupin meals to boost long-chain omega-3 had no negative effect on the meat quality parameters measured in this trial. However, sire breed significantly affects fat scores and eye muscle size, as exemplified by the East Friesian sired lambs slaughtered at the same weight as the other four breeds being leaner with smaller eye muscle measurements but heavier muscle yields.

Key Words: Lamb, Meat Quality, Omega 3, Pasture, Canola, Lupin, Lamb Meat, Human Nutrition, Red Meat, Eye Muscle, Carcass, FADS2, FABP4

Introduction

The high levels of red meat and other animal derived foods in modern western diets make it a major contributor of calories and nutrients. The intake of red meats is increasing globally as affluence increases in developing nations and is set to nearly double by 2020 (Myers and Kent, 2003). As a result, there is growing interest globally to investigate potential methods to increase long-chain polyunsaturated fatty acid (LC-PUFA) content in red meats with a focus on long-chain omega-3 (LC omega-3) to help combat potential population increases in chronic cardiovascular disease (Givens *et al.*, 2006; Kris-Etherton *et al.*, 2002; Ruxton *et al.*, 2007; Yach *et al.*, 2004). In Australia, red meat, poultry and game contribute 43% of LC omega-3 intake which is almost on par with seafood (48%) despite having significantly lower LC omega-3 content (Howe *et al.*, 2006). This indicates significantly more red meat is consumed than LC omega-3 rich seafood in Australia (Howe *et al.*, 2006). The average Australian consumes 10.4 kg of sheep meat per annum making it a significant component of the Australian diet (MLA, 2014). Therefore focusing on improving the LC omega-3 content in red meat has the potential to significantly boost the intake of these health benefiting oils by Australians (Kitessa *et al.*, 2010; Knight *et al.*, 2012).

Sheep farming is a key industry in Australian agriculture with a historical focus on wool production. In the last few decades, sheep meat has gained more popularity both domestically and internationally, which has seen a shift in farming systems, breeding programs and enterprise structure within the industry (Pethick *et al.*, 2006). The majority of sheep meat production involves mating a Merino dam with a terminal sire allowing wool production and meat production to co-exist in the same enterprise (Gardner *et al.*, 2010b). There are many meat quality parameters that could be

enhanced through genetic and production system improvements such as the enhancement of LC omega-3, however, their effects on meat quality is not well documented (Pethick *et al.*, 2006; Knight *et al.*, 2012).

The previous chapter (Chapter 2) demonstrated that supplementation of lambs with canola and lupin increased LC omega-3 content of the eye muscle and the impact of relocation due to drought conditions onto actively growing pastures in northern Tasmania did improve LC omega-3 content.

Chapter 3 hypothesises that improved long-chain omega-3 content in lamb meat will not have a negative impact on meat quality traits. This Chapter focuses on the meat quality from all animals across all treatments to investigate if any adverse meat quality interactions occurred due to attempts to enhance intramuscular LC omega-3 content.

Materials and methods

Animals and experimental design

All animals and procedures utilised in this study had the University of Tasmania Animal Ethics approval (A0009811) and were conducted in accordance with the 1993 Tasmanian Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. A half-sib experimental design was utilised in this study. Five top-EBV rams acquired from Tasmanian Sheep Stud Breeders comprising Dorset, Texel, White Suffolk, East Friesian and Coopworth were mated to purebred Merino ewes at a ratio of 1:120 ewes in separate paddocks

in a commercial farming operation in the Coal River Valley, Tasmania, to generate 500 first cross prime lambs.

Animal management

Lambs were born and raised in sire groups under similar management conditions to minimise environmental variation until weaning. The lambs were marked, vaccinated and electronically tagged at 6 weeks and run as one mob within a large scale commercial farming operation. The flock was raised during a difficult season of drought. From the third trimester onwards, the animals were raised on a mixture of limited irrigated, drought-affected perennial ryegrass (*Lolium perenne*) pastures with minimal clover and were supplemented with barley. Pastures were still actively growing with irrigation but not to their typical potential and continued extreme heat reduced their vigour. Due to the prevailing drought conditions and with the irrigation water allocations about to cease, the flock was relocated to non-drought affected winter forage crops available in northern Tasmania with forage oats and fescue as basal diet with *ad libitum* access to barley grain on offer. At seven months of age, a representative sub sample of 40 animals with a mean liveweight of 32 kg ± 2.2 and body condition score of 3 were relocated for a nine-week feeding trial.

The feeding trial animals were individually kept in 0.6 m x 1.2 m metabolic crates in an animal house at Cambridge, Southern Tasmania. Forty sheep comprising of 8 sheep from each of the 5 sire breeds were assigned to two supplementary feeds (canola or lupin) at two feed levels (1% or 2% of body weight) with ewes and wethers equally represented within each sire breed and treatment group. Two animals were removed during the trial for health issues which resulted in a total of 38 samples being collected.

Liveweight data

Liveweight was measured monthly using a Ruddweigh 3000XT walk over weighing electronic scale with RFID capability. RFID tags automatically collected lamb identities and weight at each weighing session and data were downloaded and collated into a single spreadsheet. Scales were calibrated every 100 days of the trial and tested routinely using a known mass during each weighing session to confirm correct indicated weight.

Slaughter and meat sample collection

Lambs were slaughtered in four batches as per MSA approved standards using the same kill team protocol. Carcasses were chilled overnight, and assessed the next day for fat score before processing in the boning room to separate the bone from muscle. The *Longissimus dorsi* and short loin muscles were weighed and measurements of the muscle area, height and width taken at the 12^{th} & 13^{th} ribs interface.

All samples of the *Longissimus dorsi* were placed on ice immediately after collection and transported to the laboratory and stored at -20°C until ready for genomic DNA and lipid extraction.

Identity confirmation

Blood sampling was by jugular venipuncture directly into vacutainers containing EDTA. Genomic DNA from blood samples was extracted using the UltraClean® -htp 96 Well BloodSpin® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA). DNA from muscle samples was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). DNA concentration of all samples was assessed using the

NanoDrop 8000 UV/VIS spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and purity of the DNA established by crosschecking the 260/280 nm ratio.

To ensure meat quality values derived from muscle were correctly matched to phenotypic measurements, blood originating from the same individual were used for sample matching as follows. DNA derived from 242 tissue samples used for meat quality assessment were genotyped for the 32 SNP panel used for paternity assignment described later in Chapter 6. Similarly, blood derived samples from all progeny (n = 416) were genotyped using the same set of SNP panel. The genetic similarity, estimated as allele sharing, was computed between each pair-wise combination of tissue and blood derived DNA sample. Allele sharing was calculated using PLINK v1.07 (Purcell *et al.*, 2007) which reports the average proportion of allele sharing as Dst. Tissue samples were assigned to their animal of origin where Dst > 0.95.

Statistical analyses

Meat Quality data were analysed for the fixed effects of sex, slaughter group, sire breed, SNP genotype, supplement and their second order interactions using mixed (PROC MIXED) model procedures (SAS, 2009), while the partial regressions of sire and herd were fitted as random effects. Least square means of fixed effects were obtained and tested for significance using the Tukey-Kramer adjustment test of paired values for orthogonal contrasts.

The full model was

$$Y_{ijklm} = G_i + SG_j + SB_k + SNP_l + SU_m + (GSG)_{ij} + (GSB)_{ik} + (GSNP)_{il} + (SGSB)_{jk} + (SGSNP)_{jl} + (SBSNP)_{kl} + (GSU)_{im} + (SGSU)_{jm} + (SBSU)_{km} + (SNPSU)_{lm} + b_1(S - \overline{S})^2 + b_2(H - \overline{H})^2 + e_{ijklm_n}$$

where Y_{ijklm} is the $_{ijklm}$th observation of the dependent meat quality trait with fixed effects of G_i of ith Gender (i=1,2), SG_j of jth slaughter group (j=1,2,3,4), SB_k of kth sire breed (k=1,2,3,4,5), SNP_l of lth SNP genotype (l=1,2), of the mth supplement (m=1,2), first order interaction effects $(GSG)_{ij}$, $(GSB)_{ik}$, $(GSNP)_{il}$, $(SGSB)_{jk}$, $(SGSNP)_{jl}$, $(SBSNP)_{kl}$, $(GSU)_{im}$, $(SGSU)_{jm}$, $(SBSU)_{km}$ and $(SNPSU)_{lm}$ of gender and slaughter group, gender and sire breed, gender and SNP genotype, slaughter group and sire breed, slaughter group and SNP genotype and sire breed, SNP genotype, gender and supplement, slaughter group and supplement, sire breed and supplement and SNP genotype and supplement, respectively. b_1 and b_2 are partial regression coefficients of sire and herd respectively, $b_1(S-\overline{S})^2$ and $b_1(H-\overline{H})^2$ fitted as random effects, and e_{ijklm} is a residual error term normally and independently distributed. All non-significant interactions were later removed from the final model.

Results

Table 3.1 Test of significance (P-values) for factors influencing Longissimus dorsi meat quality of first cross Merino sheep.

	Hot carcass weight	GR fat score	SC fat mm	Eye muscle width	Eye muscle height	Eye muscle area	Short loin muscle weight	Short loin bone weight	Short loin total weight
Sire	0.348	0.106	0.0001***	0.173	0.0001***	0.0001***	0.409	0.0251**	0.456
Sex	0.323	0.015	0.0001***	0.336	0.477	0.243	0.547	0.339	0.824
FABP4_SNP_1	0.591	0.228	0.629	0.127	0.336	0.124	0.792	0.821	0.496
FABP4_SNP_2	0.812	0.190	0.116	0.512	0.418	0.281	0.322	0.494	0.424
FADS2	0.619	0.247	0.958	0.612	0.636	0.148	0.051	0.604	0.085
Slaughter group	0.258	0.0007***	0.215	0.0274**	0.104	0.0002***	0.827	0.118	0.678
Supplement	0.376	0.423	0.005	0.0053*	0.741	0.394	0.930	0.241	0.971

*P<0.05, **P<0.01, ***P<0.001,

Lambs sired by Dorset and White Suffolk had significantly (P=0.001) more subcutaneous fat depth at the 12th/13th rib compared to Coopworth, Texel and East Friesian (Table 3.2). Eye muscle area (P= 0.003) and height (P=0.045) were significantly smaller in East Friesian, however, short loin muscle weight was not significantly different between the sire breeds (Table 3.2). Short loin bone weight was significantly (P=0.025) heavier in White Suffolk with average bone weights 100g heavier than observed for the four other breeds (Table 3.2).

Table 3.2 Mean meat quality parameters and standard errors in first cross Merino sheep (n=287).

	Sire Breed (287)				
	CW (40)	*EF (46)*	*DO (81)*	*TX (56)*	*W S (64)*
Hot carcass weight (Kg)	19.7 ± 0.3	20.1 ± 0.3	20.4 ± 0.3	19.8 ± 0.3	20.8 ± 0.4
GR fat score	2.9 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.5 ± 0.1	2.9 ± 0.1
Subcutaneous fat (mm)	22.6 ± 1.0	20.3 ± 0.8	25.7 ± 0.6	22.8 ± 0.7	24.9 ± 0.9
Eye muscle width (mm)	59.9 ± 0.8	59.8 ± 0.6	59.8 ± 0.5	60.5 ± 0.8	61.0 ± 0.5
Eye muscle height (mm)	30.3 ± 0.5	27.7 ± 0.5	31.4 ± 0.3	31.4 ± 0.5	30.2 ± 0.5
Eye muscle area (cm^2)	16.7 ± 0.4	15.5 ± 0.3	17.7 ± 0.3	18.0 ± 0.3	17.3 ± 0.3
Short loin muscle (Kg)	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Short loin bone (Kg)	1.4 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.2	1.5 ± 0.0
Short loin total weight (Kg)	1.9 ± 0.2	1.9 ± 0.1	2.4 ± 0.3	1.9 ± 0.1	2.2 ± 0.1

Slaughter group significantly affected a number of variables in the experiment. Slaughter group 4, the largest batch of lambs slaughtered at a heavier liveweight, had the heaviest carcass and demonstrated overall best meat quality (Table 3.3). Slaughter group 4 had significantly higher (P<0.001) GR fat score compared to the other three slaughter groups (Table 3.3). Eye muscle width in slaughter group 1 was significantly smaller (P<0.03) and similarly eye muscle area was significantly (P<0.001) smaller in this group (Table 3.3).

Table 3.3 Mean meat quality parameters +/- standard error by slaughter group (n=315).

	Slaughter Group#											
	1(47)			2 (60)			3 (38)			4 (170)		
Hot carcass weight (Kg)	19.7	±	0.2	18.8	±	0.2	18.5	±	0.3	21.2	±	0.2
GR Fat score	2.3	±	0.1	2.1	±	0.0	2.3	±	0.1	3.3	±	0.0
Subcutaneous Fat (mm)	25.9	±	0.9	24.4	±	0.8	27.1	±	1.6	22.0	±	0.3
Eye muscle width (mm)	56.8	±	0.6	59.4	±	0.6	62.2	±	1.0	61.2	±	0.4
Eye muscle height (mm)	30.7	±	0.5	31.4	±	0.4	27.4	±	0.7	30.6	±	0.2
Eye muscle area (cm^2)	15.8	±	0.3	17.7	±	0.3	16.8	±	0.5	17.5	±	0.2
Short loin muscle (Kg)	0.6	±	0.0	1.4	±	0.8	0.6	±	0.0	0.7	±	0.0
Short loin bone (Kg)	1.3	±	0.0	1.3	±	0.0	1.4	±	0.1	1.5	±	0.0
Short loin total weight (Kg)	1.9	±	0.0	2.7	±	0.8	2.2	±	0.1	2.2	±	0.1

Slaughter Group one=April 2008 fastest growth, group two= May 2008 moderate growth, group three= June 2008 feeding trial and group four=August 2008 slowest growth

Females had significantly (P<0.00.1) more subcutaneous fat compared to males across all sire breeds (Table 3.4). Ewe GR Fat scores (Scale 1-5) were 0.2 points higher than for wethers.

Supplementation with lupin or canola meal did significantly affect a number of meat quality variables measured. Animals fed lupins had significantly (P<0.01) shorter eye muscle height (Table 3.4). Supplementation of lambs with canola at both levels significantly (P=0.006) increased eye muscle width by 3.3mm (Table 3.4). All supplemented animals recorded significantly (P<0.005) lower GR fat score compared to animals reared on pastures. Feeding trial animals were sacrificed at a fixed time point rather than target carcass weight, yet measured comparable eye muscle width despite mean lower hot carcass weights. There were no significant effects based on SNP genotypes for any meat quality parameters measured (Table 3.5) but is discussed in more depth in Chapter 5.

Table 3.4 Meat quality means ±S.E. of first cross Merino sheep grouped by sex and supplement (n=347).

	Sex (309)		Supplement (38)				
	Male (163)	Female (146)	1% Canola (10)	2% Canola (9)	1% Lupin (10)	2% Lupin (9)	Pasture (316)
Hot carcass weight (kg)	20.3 ± 0.2	20.1 ± 0.2	18.8 ± 0.5	19.6 ± 0.6	18.2 ± 1.0	17.4 ± 0.2	20.2 ± 1.1
GR fat score	2.7 ± 0.1	2.9 ± 0.1	2.2 ± 0.1	2.4 ± 0.2	2.4 ± 0.2	2.1 ± 0.1	2.8 ± 0.1
Subcutaneous Fat (mm)	23.2 ± 0.5	24.1 ± 0.5	25.3 ± 2.1	27.8 ± 1.8	32.8 ± 3.4	20.9 ± 3.5	23.7 ± 1.8
Eye muscle width (mm)	60.0 ± 0.4	60.6 ± 0.5	64.3 ± 1.9	63.0 ± 2.2	61.9 ± 2.6	59.0 ± 1.8	60.3 ± 2.0
Eye muscle height (mm)	30.4 ± 0.3	30.4 ± 0.3	26.6 ± 1.9	27.8 ± 0.7	28.1 ± 1.2	26.6 ± 1.4	30.4 ± 1.8
Eye muscle area (cm^2)	17.1 ± 0.2	17.3 ± 0.2	16.2 ± 0.6	16.9 ± 0.7	17.4 ± 1.7	16.6 ± 1.2	17.2 ± 0.5
Short loin muscle (kg)	0.6 ± 0.5	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.9 ± 0.0
Short loin bone (kg)	1.4 ± 0.0	1.4 ± 0.0	1.5 ± 0.1	1.4 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	1.4 ± 0.1
Short loin total weight (kg)	2.3 ± 0.3	2.2 ± 0.2	2.3 ± 0.1	2.4 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.3 ± 0.2

Table 3.5 Mean +/- SE for meat quality traits grouped by genotype for the three SNP loci investigated. Number of animals per genotype is denoted in brackets.

	FABP 4 SNP1 (284)			FABP 4 SNP2 (292)		FADS2 (288)		
Trait	***AA (190)***	***AG (44)***	***GG (50)***	***AG (48)***	***GG (244)***	***CC (228)***	***CT (53)***	***3 (203)***
Hot carcass weight (Kg)	20.1 ± 0.2	20.4 ± 0.3	20.8 ± 0.4	20.2 ± 0.3	20.2 ± 0.2	20.3 ± 0.1	20.1 ± 0.5	19.9 ± 0.9
GR fat score	2.8 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.0	2.8 ± 0.0	2.8 ± 0.1	2.5 ± 0.2
Subcutaneous fat (mm)	23.2 ± 0.4	24.2 ± 1.2	23.8 ± 0.7	23.4 ± 0.9	23.5 ± 0.4	23.2 ± 0.4	24.9 ± 1.1	25.7 ± 2.2
Eye muscle width (mm)	60.6 ± 0.4	61.0 ± 0.7	59.5 ± 0.7	60.2 ± 0.7	60.4 ± 0.4	60.1 ± 0.4	61.3 ± 0.6	59.3 ± 1.0
Eye muscle height (mm)	30.2 ± 0.2	30.4 ± 0.6	31.1 ± 0.4	30.1 ± 0.6	30.4 ± 0.2	30.3 ± 0.2	30.5 ± 0.4	30.9 ± 0.7
Eye muscle area (cm^2)	17.0 ± 0.2	17.3 ± 0.4	18.3 ± 0.4	17.1 ± 0.4	17.2 ± 0.2	17.2 ± 0.2	17.1 ± 0.3	19.6 ± 1.6
Short loin muscle (Kg)	1.0 ± 0.4	0.6 ± 0.0	0.6 ± 0.0	2.5 ± 1.9	0.6 ± 0.0	0.6 ± 0.0	2.0 ± 1.4	0.6 ± 0.0
Short loin bone (Kg)	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.3 ± 0.1
Short loin total weight (Kg)	2.3 ± 0.3	2.1 ± 0.1	2.6 ± 0.4	3.1 ± 1.1	2.1 ± 0.1	2.1 ± 0.1	3.0 ± 0.9	2.2 ± 0.1

Short loin bone weight was not collected on every sample and was only measured to 1 decimal place.

Table 3.6 Abbreviated table demonstrating assignment of sample matching using average proportion of allele sharing between tissue and blood samples where Dst > 0.95 cells are colour coded green for sample matching and red for 100% blood to DNA allele sharing.

Blood DNA Sample Identity	Tissue DNA Sample Identity									
	153	***154***	***155***	***156***	***157***	***158***	***159***	***160***	***161***	***162***
B1	0.633	0.714	0.650	0.607	0.600	0.638	0.696	0.600	0.696	0.696
B10	0.661	1.000	0.565	0.707	0.673	0.683	0.638	0.629	0.652	0.724
B100	0.645	0.690	0.548	0.621	0.808	0.633	0.759	0.710	0.783	0.776
B11	0.554	0.673	0.643	0.714	0.740	0.643	0.796	0.607	0.690	0.731
B12	0.548	0.707	0.677	0.690	0.558	0.717	0.724	0.613	0.717	0.759
B13	0.638	0.759	0.690	0.714	0.600	0.690	0.714	0.707	0.818	0.778
B14	0.574	0.640	0.593	0.673	0.625	0.611	0.981	0.685	0.690	0.700
B15	0.690	0.778	0.690	0.661	0.620	0.707	0.732	0.655	0.762	0.759
B17	0.710	0.741	0.645	0.690	0.712	0.667	0.741	0.677	0.761	0.776

The allele matrix approach to matching muscle tissue and blood derived DNA samples confirmed the identity of 236 samples (Table 3.6). Only 50 blood samples could not be matched to a tissue sample indicating recording errors did occur and had been addressed using the allele matrix approach and is discussed further in Chapters 5 & 6.

Discussion

The experimental design was focused on enhancing LC omega-3 content within the constraints of a commercial farming operation and animals were slaughtered according to their growth rates rather than set age at slaughter except the feeding trial supplemented animals. The model of slaughtering at liveweight has been used in numerous studies on meat quality in last forty years to asses breed potential and feed regimes (Atkins and Thompson, 1979; Ponnampalam *et al.*, 2008; Ponnampalam *et al.*, 2007; Scales *et al.*, 2000). Feeding trial animals were selected at the start of the trial at a starting weight that should have led to a liveweight of 44

kg plus at the conclusion of the trial based on industry figures of expected growth rates. The difficult season of drought and relocation had unforeseen impacts on growth rates, however, all animals slaughtered showed no sign of poor meat quality, but a depressed growth rate occurred when the animals were relocated. The majority of Australian sheep meat producers sell on a grid pricing structure requiring them to meet specific market expectations to achieve premium prices for their livestock and variation outside of the grid result in significant price penalties for the producer so consistent meat quality results are essential to the industry (Duddy *et al.*, 2005).

Overall, carcass weights were not significantly affected by any of the variables tested in the experiment as outlined in Table 3.1. This is due to the experimental design where animals were killed based on their growth rates and time taken to reach the target carcass weight of 20 kg. However, it was apparent that lambs from Dorset and White Suffolk sires made up the majority of the first two slaughter groups indicating they were growing on average, significantly faster than their counterparts sired by Texel, Coopworth and East Friesian. The fast growth rate of Dorset and White Suffolk compared to the other three sire breeds has been reported in a number of other trials and our results are in agreement with previous observations that Dorset and White Suffolk reach target carcass weights quicker than East Friesian, Coopworth and Texel when raised under the same conditions (Fogarty *et al.*, 2005; Ponnampalam *et al.*, 2007; Scales *et al.*, 2000; Annett *et al.*, 2011; Fogarty and Mulholland, 2012). The last slaughter group of animals was the largest group (n=170) and slaughter occurred at 10 months of age with slightly higher mean hot carcass weights. The animals in slaughter group 4 had been relocated due to drought conditions and growth was stagnant for seven weeks until positive growth occurred again. Group 4 carcass weights were consistent with weights reported by

Scales (2000) and Ponnampalam *et al.* (2007) for first cross Texel, Dorset and East Friesian lambs at eight months of age and slightly light for animals at ten months of age. The implication is that drought conditions and relocation negatively impacted on growth and carcass yield.

Indexing carcasses to a standard weight to compare fat scores has been reported to introduce bias into the comparison of animals sacrificed at different dates; the influence of season has been shown to override any interactions and given the difficult season encountered in this study, the data conformed to expectation (Scales *et al.*, 2000; Ponnampalam *et al.*, 2007). The GR fat score showed a trend of increasing with time and slaughter date significantly affected this score with the last slaughter group being 1 score (out of 5) higher than the first (Table 3.2). It is widely reported that animals with rapid growth rates typically have a lower affinity to depositing fat with a preference to building skeleton and muscle and our GR Fat results are in agreement with these findings (Afolayan *et al.*, 2007; Annett *et al.*, 2011; Black, 1983; Speijers *et al.*, 2009).

Animals sired by the East Friesian had significantly ($P<0.001$) smaller eye muscle height and area, however, short loin yield was not significantly ($P<0.05$) affected. East Friesian is a dairy breed and often used in meat production systems to boost fertility and milk production in breeding ewes rather than meat yield. Similar eye muscle results in East Friesian first cross lambs in our study have been previously reported (Afolayan *et al.*, 2007; Fogarty *et al.*, 2005; Scales *et al.*, 2000). As animals matured, eye muscle area increased. This increase is likely due to growth preferences as animals transitioned from rapid bone and muscle growth to a bias towards muscle and fat deposition (Ponnampalam *et al.*, 2008). Slaughter date was

a significant (P<0.001) variable for eye muscle area with slaughter group one having the smallest eye muscle area. However, the lack of significance for slaughter date (P<0.05) affecting short loin bone weight and muscle yield suggests that despite a change in physical size, animals at a target carcass weight will all yield similar eye muscle by weight and younger animals will have a flatter and smaller area muscle compared to a slower maturing animal.

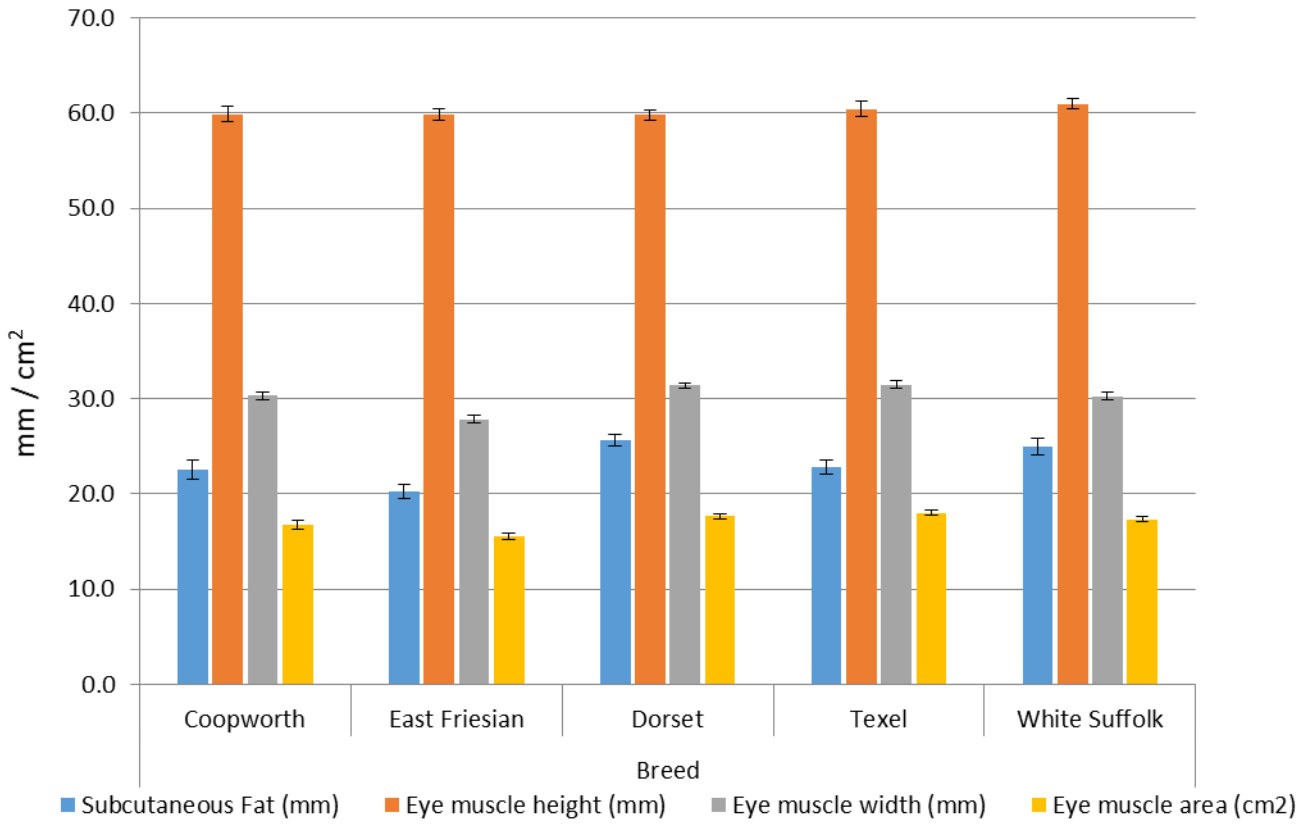

Figure 3.1 Mean meat quality parameters for the Longissimus dorsi grouped by sire breed (n=315).

It has been reported that Texel and East Friesian have higher maturity weight than Dorset or White Suffolk and therefore, when measured at the same carcass weight, slower maturing animals will have a lower fat score than fast maturing breeds.

(Annett *et al.*, 2011; Gardner *et al.*, 2010a; Scales *et al.*, 2000). This effect was observed in this trial with sire being a significant (P<0.001) source of variation influencing subcutaneous fat. Dorset and White Suffolk had the highest fat score values and East Friesian were the lowest based on a 20kg average hot carcass weight (Figure 3.1). It is worth noting GR Fat was not affected by sire, however, subcutaneous fat was measured in mm and offered higher resolution compared to the 1-5 GR Fat scale.

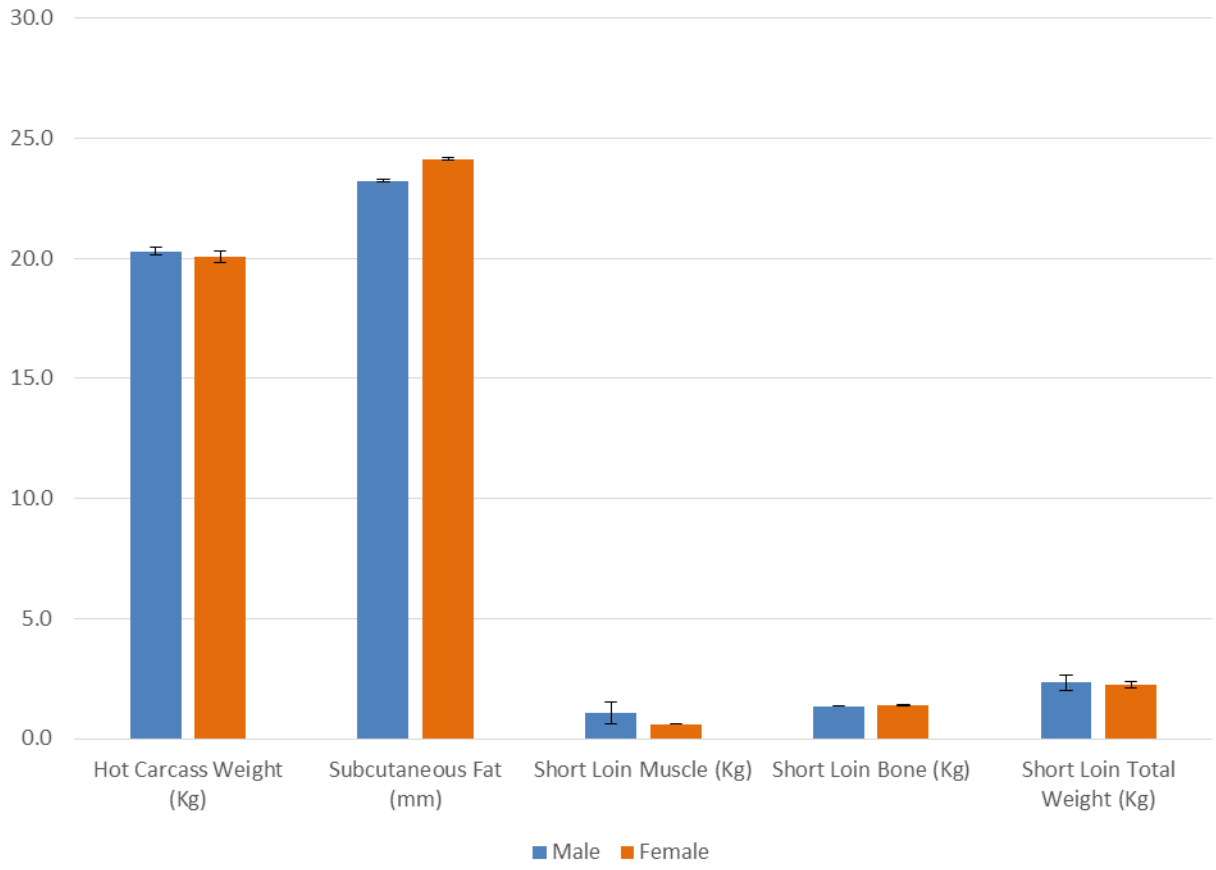

Figure 3.2 Mean meat quality parameters for the Longissimus dorsi grouped by sex (n=315).

Females were 9 mm fatter than males (Figure 3.2) and this observation may be attributed to a mixture of growth rate, sire and slaughter date. Various studies have reported significant impact of sex on intramuscular and subcutaneous fat deposition

(Holman et al 2014; Flakemore et al. 2014a, 2014b; Dervishi et al. 2012; McPhee et al. 2008; Pethick et al. 2004). Sexual dimorphism between ewes and wethers in terms of GR fat score is linked to hormonal variation as females generally have the propensity for accelerated early fat deposition compared to males where muscular accretion is more pronounced. Furthermore, the mechanism for the effect of sex on GR Fat score is thought to be dictated by cellular signal transductions and their subsequent impacts on enzymatic pathways linked to lipogenesis. This is an area that needs further research to unpack the underlying biological and molecular mechanisms.

It has been observed that ewes are fatter than wethers of the same age at slaughter (Annett *et al.*, 2011; Black, 1983; Fogarty and Mulholland, 2012; Ponnampalam *et al.*, 2008; Ponnampalam *et al.*, 2007; Kitessa *et al.*, 2010). Wether lambs grow faster than ewes of the same age and therefore reach target slaughter weight sooner (Black, 1983). In a commercial situation, it would be expected that wethers would be slaughtered earlier and potentially have leaner carcasses due to the preference to growth and lean muscle deposition compared to ewes (Black, 1983; Fogarty and Mulholland, 2012).

Feed treatments to boost long-chain omega-3 did not negatively impact on any of the meat quality variables measured. Noteworthy, animals fed 1% lupins in the feeding trial had significantly ($P<0.005$) larger eye muscle area, however, the standard error was ±1.7 cm and with the small sample size (n=10) used, further investigation is needed to confirm this result. The animals in the supplementary feeding trial were killed on a fixed date rather than growth rates to hot carcass weight and this increased the overall variation within breeds but was still not significant ($P<0.05$).

Conclusion

Slaughtering animals according to growth rates allowed for clear genetic distinctions of meat quality traits across sire breeds. Dorset and White Suffolk demonstrated a preference for rapid growth rates producing larger carcasses with higher fat scores than the East Friesian breed which was significantly leaner than the other breeds tested. The use of canola and lupins as supplements to enhance long-chain omega-3 did not have a negative impact on the meat quality and lupins improved the eye muscle size compared to all other treatments. The drought conditions experienced during the trial did have an impact on the growth rates of the flock however all animals reached their target carcass weights. The results show that techniques to improve long-chain omega-3 content will not adversely affect profitability of a sheep meat business through meat quality penalties. The sex effect requires further investigation to fully understand and the effects of rearing on lush green pasture from birth to slaughter would provide deeper insight into the potential to boost long-chain omega-3 and meat quality in Australian lamb.

References

Afolayan, R. A., Fogarty, N. M., Gilmour, A. R., Ingham, V. M., Gaunt, G. M. & Cummins, L. J. (2008). Genetic correlations between reproduction of crossbred ewes and the growth and carcass performance of their progeny. *Small Ruminant Research* 80(1–3): 73-79.

Afolayan, R. A., Fogarty, N. M., Ingham, V. M., Gilmour, A. R., Gaunt, G. M., Cummins, L. J. & Pollard, T. (2007). Genetic evaluation of crossbred lamb production. 3. Growth and carcass performance of second-cross lambs. *Australian Journal of Agricultural Research* 58(5): 457-466.

Annett, R. W., Carson, A. F., Dawson, L. E. R. & Kilpatrick, D. J. (2011). Effects of dam breed and dietary source of n-3 polyunsaturated fatty acids on the growth and carcass characteristics of lambs sourced from hill sheep flocks. *Animal* 5(7): 1023-1035.

Atkins, K. D. & Thompson, J. M. (1979). Carcass characteristics of heavyweight crossbred lambs 1. Growth and carcass measurements. *Australian Journal of Agricultural Research* 30(6): 1197-1205.

Aurousseau, B., Bauchart, D., Faure, X., Galot, A. L., Prache, S., Micol, D. & Priolo, A. (2007). Indoor fattening of lambs raised on pasture. Part 1: Influence of stall finishing duration on lipid classes and fatty acids in the longissimus thoracis muscle. *Meat Science* 76(2): 241-252.

Black, J. L. (1983).Growth and development of lambs. In *Sheep production.*, 21-58: Butterworths; London; UK.

D'Alessandro, A. G., Maiorano, G., Ragni, M., Casamassima, D., Marsico, G. & Martemucci, G. (2013). Effects of age and season of slaughter on meat production of light lambs: Carcass characteristics and meat quality of Leccese breed. *Small Ruminant Research* 114(1): 97-104.

Dervishi, E., Joy, M., Alvarez-Rodriguez, J., Serrano, M. & Calvo, J. H. (2012). The forage type (grazing versus hay pasture) fed to ewes and the lamb sex affect fatty acid profile and lipogenic gene expression in the longissimus muscle of suckling lambs1. *Journal of Animal Science* 90(1): 54-66.

Duddy, G., Hegarty, R. S. & White, A. (2005).Managing for lamb production. In *Sheep Production*, 9-1 - 9-20 (Ed D. Cottle). Armidale, Australia: School of Rural Science and Agriculture University of New England.

Flakemore, A.R., Balogun, R.O., McEvoy, P.D., Malau-Aduli, B.S., Nichols, P.D., Malau-Aduli, A.E.O., (2014). Genetic variation in intramuscular fat of prime lamb supplemented with varying concentrations of degummed crude canola oil. *International Journal of Nutrition and Food Sciences*, 3 (3): 203-209, doi:10.11648/j.ijnfs.20140303.22

Flakemore, A.R., McEvoy, P.D., Balogun, R.O., Malau-Aduli, B.S., Nichols, P.D., Malau-Aduli, A.E.O., (2014a). Degummed crude canola oil supplementation affects fat depot melting points in purebred and first-cross Merino sheep. *Animal and Veterinary Sciences*, 2: 75-80.

Fogarty, N. M., Ingham, V. M., Gilmour, A. R., Cummins, L. J., Gaunt, G. M., Stafford, J., Edwards, J. E. H. & Banks, R. (2005). Genetic evaluation of crossbred lamb production. 2. Breed and fixed effects for post-weaning growth, carcass, and wool of first-cross lambs. *Australian Journal of Agricultural Research* 56(5): 455-463.

Fogarty, N. M. & Mulholland, J. G. (2012). Growth and carcass characteristics of crossbred lambs in various production systems. *Animal Production Science* 52(5): 373-381.

Gardner, G. E., Williams, A., Siddell, J., Ball, A. J. & Mortimer, S. (2010a). Using Australian Sheep Breeding values to increase lean meat yield percentage. *Animal Production Science* 50(11--12): 1098-1106.

Gardner, G. E., Williams, A., Siddell, J., Ball, A. J., Mortimer, S., Jacob, R. H., Pearce, K. L., Edwards, J. E. H., Rowe, J. B. & Pethick, D. W. (2010b). Using Australian Sheep Breeding Values to increase lean meat yield percentage. *Animal Production Science* 50(11-12): 1098-1106.

Givens, D. I., Khem, K. E. & Gibbs, R. A. (2006). The role of meat as a source of n-3 polyunsaturated fatty acids in the human diet. *Meat Science* 74(1): 209-218.

Holman, B.W.B., Flakemore, A.R., Kashani, A., Malau-Aduli, A.E.O., (2014). Spirulina supplementation, sire breed, sex and basal diet effects on lamb intramuscular fat percentage and fat melting points. *International Journal of Veterinary Medicine: Research & Reports*, Vol. 2014, Article ID 263951, doi: 10.5171/2014.263951, 9 pages.

Howe, P., Meyer, B., Record, S. & Baghurst, K. (2006). Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition* 22(1): 47-53.

Kitessa, S., Liu, S. M., Briegel, J., Pethick, D., Gardner, G., Ferguson, M., Allingham, P., Nattrass, G., McDonagh, M., Ponnampalam, E. & Hopkins, D. (2010). Effects of intensive or pasture finishing in spring and linseed supplementation in autumn on the omega-3 content of lamb meat and its carcass distribution. *Animal Production Science* 50(2): 130-137.

Knight, M. I., Daetwyler, H. D., Hayes, B. J., Hayden, M. J., Ball, A. J., Pethick, D. W. & McDonagh, M. B. (2012). Discovery and trait association of single nucleotide polymorphisms from gene regions of influence on meat tenderness and long-chain omega-3 fatty acid content in Australian lamb. *Animal Production Science* 52(6--7): 591-600.

Kris-Etherton, P. M., Harris, W. S., Appel, L. J. & Nutrition, C. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 106(21): 2747-2757.

McPhee, M.J., Hopkins, D.L., Pethick, D.W., (2008). Intramuscular fat levels in sheep muscle during growth. *Australian Journal of Experimental Agriculture*, 48: 904-909.

MLA (2010).Australian sheep meat industry overview. In *Australian sheep meat industry projections 2010*: Meat and Livestock Australia.

Myers, N. & Kent, J. (2003). New Consumers: The influence of affluence on the environment. *Proceedings of the National Academy of Sciences of the United States of America* 100(8): 4963-4968.

Pethick, D. W., Harper, G. S., Oddy, V. H., (2004). Growth, development and nutritional manipulation of marbling in cattle: A review. *Australian Journal of Experimental Agriculture* 44, 705-715.

Pethick, D. W., Banks, R. G., Hales, J. & Ross, I. R. (2006). *Australian prime lamb - a vision for 2020.* Armidale, Australia: Australian Sheep Industry CRC.

Ponnampalam, E. N., Butler, K. L., Hopkins, D. L., Kerr, M. G., Dunshea, F. R. & Warner, R. D. (2008). Genotype and age effects on sheep meat production. 5. Lean meat and fat content in the carcasses of Australian sheep genotypes at 20-, 30- and 40-kg carcass weights. *Australian Journal of Experimental Agriculture* 48(6-7): 893-897.

Ponnampalam, E. N., Hopkins, D. L., Butler, K. L., Dunshea, F. R. & Warner, R. D. (2007). Genotype and age effects on sheep meat production 2. Carcass quality traits. *Australian Journal of Experimental Agriculture* 47(10): 1147-1154.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J. & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* 81(3): 559-575.

Ruxton, C. H. S., Reed, S. C., Simpson, M. J. A. & Millington, K. J. (2007). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *Journal of Human Nutrition and Dietetics* 20(3): 275-285.

SAS (2009).SAS® Software. Cary, NC, USA: SAS Institute Inc.

Scales, G. H., Bray, A. R., Baird, D. B., O'Connell, D. & Knight, T. L. (2000). Effect of sire breed on growth, carcass, and wool characteristics of lambs born to Merino ewes in New Zealand. *New Zealand Journal of Agricultural Research* 43(1): 93-100.

Speijers, M. H. M., Carson, A. F., Dawson, L. E. R. & Gordon, A. W. (2009). Effects of genotype and plane of nutrition on growth and carcass characteristics of lambs from hill sheep systems. *Animal* 3(9): 1232-1245.

Yach, D., Hawkes, C., Gould, C. L. & Hofman, K. J. (2004). The global burden of chronic diseases - Overcoming impediments to prevention and control. *Jama-Journal of the American Medical Association* 291(21): 2616-2622.

Chapter 4

The effects of drought and relocation on l*ongissimus dorsi* muscle fatty acid content in first-cross Merino prime lambs.

Abstract

Australian consumers are becoming increasingly aware of the health benefits of long-chain (≥C_{20}) omega-3 polyunsaturated fatty acids (LC omega-3) - eicosapentaenoic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6ω3). This study investigated the effects of drought-affected pasture and lamb relocation on the LC omega-3 content in 365 first cross Merino lambs sired by Texel, East Friesian, Dorset, Coopworth and White Suffolk rams. To eliminate maternal variation, Merino ewes were joined to these sire breeds to produce the F_1 progeny. The flock was raised on irrigated improved pasture before relocation to northern Tasmania which was not in drought. Animals were slaughtered on reaching 44.5 kg liveweight in 3 slaughter groups. Total muscle fatty acid contents of the three slaughter groups were: 1=1394 mg/100 g, 2=1335 mg/100 g, 3=1969 mg/100 g. There was a lower mean accumulation of intramuscular fatty acid content (IMF) in slaughter groups 1 and 2 compared to slaughter group 3 suggesting that groups 1 and 2 favoured muscle growth over fatty acid storage and IMF increased with time. Percentages of IMF were 2.5%, 2.3% and 3.5% for slaughter groups 1, 2 and 3, respectively. Muscle α-linolenic acid (ALA, 18:3ω3) contents for the three slaughter groups were 1=14.9 mg/100 g, 2=13.3 mg/100 g and 3=30.1 mg/100 g. LC omega-3, EPA + DHA, content was comparatively lower in slaughter groups 1 and 2 than in slaughter group 3 (7.2 mg/100 g, 7.8 mg/100 g and 15.2 mg/100 g, respectively). The ω6 to ω3 ratio for the first two kills were 2.4:1 and 2.7:1 and dropping to 1.4:1in the third slaughter

group. Texel sired lambs had a significantly lower (P=0.045) DHA intramuscular content than other sire breeds. None of the animals reached the claimable dietary “source” level of LC omega-3 (30 mg EPA + DHA / 100 g). Flock relocation to non-drought affected pastures did not result in rapid compensatory growth, although the heath attributes of intramuscular FA profiles did improve but still did not meet dietary “source” levels.

Key Words: Lamb, Fatty Acids, Omega 3, PUFA, Pasture, ALA, lamb meat, EPA, DHA, human nutrition, red meat, drought

Introduction

The high levels of red meat and other animal derived foods in modern western diets make it a major contributor of calories and nutrients. The intake of red meats is increasing globally as affluence increases in developing nations and is set to nearly double by 2020 (Myers and Kent, 2003). In Australia, an average of 46.5 kg of red meat is consumed each year, of which 10.8 kg is lamb (MLA, 2011). It has been suggested that a diet rich in red meats may increase the risk of cardiovascular disease and colon cancer, which has in turn led to a negative perception of the role of red meat in health and well-being (McAfee *et al.*, 2010b). One of the major concerns has been related to saturated fatty acids (SFA). Ruminants produce fat that is relatively high in SFA due to biohydrogenation of consumed polyunsaturated fatty acids (PUFA) in the rumen which is exacerbated by rumen acidification when fed grains (Bauman *et al.*, 2003; Noble, 1981). The adipose tissue on red meat is essentially the trimmable fat and the greatest determinant of SFA (Williams and Droulez, 2010). Consumers in Australia today are eating heavily trimmed, leaner

cuts of red meat which contain healthier intramuscular fat (IMF) fatty acid profiles than previously consumed. As such, red meat is no longer a substantial source of SFA in the Australian diet (Williams and Droulez, 2010).

Studies on the dietary intake of PUFA, specifically long chain ($\geq C_{20}$) omega-3 polyunsaturated fatty acids (LC omega-3), have indicated that Australians are not consuming enough of these healthful fatty acids to maximise their relevance in healthy cardiovascular function, improved mental health and infant brain development (Howe *et al.*, 2006). Alongside low LC omega-3 intake, western diets generally contain a high $\omega 6{:}\omega 3$ ratio in the order of 15:1 which is considered unfavourable for good health and a ratio closer to 5:1 such as occurs in many eastern diets, is more desirable(Givens *et al.*, 2006; McAfee *et al.*, 2010b; Trautwein, 2001).

Marine based sources of LC omega-3 are under considerable strain with industrial fishing and other industries competing for the LC omega-3 rich oils. Therefore, alternative dietary sources are of great interest to both industry and consumers (Nichols *et al.*, 2010). As a result of the global search for new sources of the LC omega-3 resource and escalating affluence in developing countries, alternative sources are being investigated to mitigate pressure on already strained health systems and improved population health both in developing and developed countries.

Increased consumer awareness of the health benefits of LC omega-3 has seen the advent of alternative dietary sources of LC omega-3 products such as fortified bread, juice and milk along-side naturally rich dietary sources of LC omega-3 such as oily

fish. Trials into enhancing the LC omega-3 content of lamb meat have shown that feeding sheep a rumen-protected LC omega-3 source such as tuna oil can significantly raise intramuscular LC omega-3 content without negative effect on meat quality or animal performance (Kitessa *et al.*, 2001). Commercial constraints and market acceptance of rumen-protected LC omega-3 being fed to animals means that presently the most profitable proposition is utilising pasture fed lamb rather than rumen-protection.

A number of trials have shown that diet affects the LC omega-3 content of lamb and diet can be manipulated to enhance levels, however IMC of LC omega-3 has been highly varied (Mortimer *et al.*, 2010; Scerra *et al.*, 2011; Skapetas *et al.*, 2009; Voicu *et al.*, 2010; Wachira *et al.*, 2002; Aurousseau *et al.*, 2007e). The LC omega-3 - eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - are the two recognised LC omega-3 to have scientifically proven health claims. As a result, Food Standards Australia and New Zealand (FSANZ) only recognise EPA + DHA when making dietary claims for omega-3 content. The two claimable minimum levels of LC omega-3 content are 30 mg EPA + DHA /100 g for “source” and 60 mg or greater for ‘good source’ (FSANZ, 2003).

Studies on pasture based feeding of lamb to elevate EPA + DHA content have shown levels ranging from negligible through to claimable source levels, with variation between breeds being as great as within breeds (Aurousseau *et al.*, 2007e; Caparra *et al.*, 2007; Cooper *et al.*, 2004; Droulez *et al.*, 2006; Howe *et al.*, 2006; Mortimer *et al.*, 2010; Wachira *et al.*, 2002). A major influence on intramuscular LC omega-3 content in pasture reared lambs appears to be the ‘greenness’ or quality of pasture available for feeding. Lush, unstressed green pasture is a rich source of the LC omega-3 precursor fatty acid alpha-linoleic acid (ALA). In a comparison of the FA

profiles of several forage species grown under glass house conditions, ALA was the most abundant FA across species and on average, made up 62% of the fatty acid profile (Clapham *et al.*, 2005). However, studies have found that the level of ALA varied by a factor of 12 within 3 species of ryegrass under glass house conditions (Dewhurst *et al.*, 2001). It has been reported that glass house trials have generally overestimated total FA levels compared to field grown pasture samples (Dierking *et al.*, 2010). This would suggest that biotic and abiotic stresses within the field have a greater effect than plant genetics on FA levels. ALA levels in grass are reported to drop over summer as the pasture dries and studies using summer pastures and dry hay have shown a decrease in content of intramuscular ALA in lamb meat as a result (Aurousseau *et al.*, 2007e; Dierking, 2008; Elgersma *et al.*, 2003; Mortimer *et al.*, 2010).

Chapter 4 tested the hypothesis that drought affected pasture will have a negative impact on LC omega-3 content and relocation to improved lush green pasture will improve long-chain omega-3 content. The main objectives of this study were to investigate the base-line content of LC omega-3 fatty acids, and the wider FA profile of Tasmanian lamb. Further, the experimental design enabled quantification of the effect of sheep grazing heat stressed pastures to relocation to lush; non-heat stressed pastures on the fatty acid profiles of the *Longissimus dorsi* muscle in first-cross Merino prime lambs sired by five commonly used ram breeds.

Materials and methods

Animals and experimental design

A half-sib experimental design was utilised in this study. Five top-EBV rams acquired from Tasmanian Sheep Stud Breeders comprising Dorset, Texel, White Suffolk, East Friesian and Coopworth were mated to purebred Merino ewes at a ratio of 1:120 ewes in separate paddocks in a commercial farming operation in the Coal River Valley, Tasmania, to generate 500 first cross prime lambs.

Animal management

Lambs were born in sire groups under similar management conditions to minimise environmental variation until weaning. The lambs were marked, vaccinated and electronically tagged at 6 weeks and run as one mob within a large scale commercial farming operation. The flock was raised during a difficult season of drought stress. From the third trimester onwards, the animals were raised on a mixture of limited irrigated, drought-affected perennial ryegrass (*Lolium perenne*) pastures with minimal clover and were supplemented with barley. Pastures were still actively growing with irrigation, but not to their typical potential and continued extreme heat reduced their vigour and nutritional value. Due to the prevailing drought conditions and with irrigation water allocations about to cease, the flock was relocated to non-drought affected winter forage crops available in northern Tasmania with forage oats and fescue as basal diet with *ad libitum* access to barley grain on offer.

Blood Sampling

Blood sampling was by jugular venepuncture directly into vacutainers containing EDTA.

Slaughter

The prime lambs were slaughtered at the Longford commercial abattoir at 44 kg or greater slaughter weight and carcasses were chilled overnight. *Longissimus dorsi* muscle tissue samples from 365 prime lambs were collected and transported to the laboratory in ice-containing baths and stored at -20°C until ready for genomic DNA and lipid extraction.

Liveweight data

Liveweights were measured monthly using a Ruddweigh 3000XT walk over weighing electronic scale with capability of automatic scanning of lamb identity and downloading of weight data into excel spreadsheets.

Pasture Sampling

Pastures were sampled at lamb marking, weaning and monthly till relocation from South East Tasmania to Northern Tasmania. No pasture samples were collected from the northern site. Whole pasture swards were collected using 0.25 m^2 quadrats and cut to 25 mm above ground level. One quadrat per five hectares was collected across all fields the animals had access to grazing. Each sample interval was bulked together and mixed thoroughly and a 1 kg subsample collected and stored at -20°C. 100 g from each sample was freeze dried and ground for fatty acid extraction.

Fatty acid analysis

About 1 g of *Longissimus dorsi* muscle samples from the 12^{th} rib interface and approximately 1.5 g of freeze dried pasture were used for fatty acid analysis. Lipid was extracted using a modified Bligh and Dyer protocol (Bligh and Dyer, 1959). This involved a single phase extraction, $CHCl_3$/MeOH/H_2O (1:2:0.8, by vol.), followed by phase separation to yield a total lipid extract (TLE). An aliquot of the TLE was trans-

methylated in methanol: chloroform; hydrochloric acid (10:1:1, v/v/v) for 2 hours at 80°C. After addition of water, the mixture was extracted three times with hexane: dichloromethane (4:1, v/v) to obtain fatty acid methyl esters (FAME) which were concentrated under a stream of nitrogen gas. Samples were made up to a known volume with an internal injection standard (19:0 FAME) added and analysed by gas chromatography (GC) using an Agilent Technologies 7890A GC (Palo Alto, California, USA) equipped with an Supelco Equity-1 fused silica capillary column (15 m×0.1 mm). Helium was used as the carrier gas. Samples were injected, by using a split/splitless injector operated in splitless mode and an Agilent Technologies 7683B Series auto-sampler, at an oven temperature of 120 °C. After 1 minute, the oven temperature was raised to 270 °C at 10 °C per minute and finally to 300 °C at 5 °C minute which was held for 5 min. Peaks were quantified by Agilent Technologies GC ChemStation software (Palo Alto, CA, USA). GC–mass spectrometric analyses were performed on a Finnigan Thermoquest GCQ GC–mass spectrometer fitted with an on-column injector and using Thermoquest Xcalibur software (Austin, TX, USA). The GC was fitted with a capillary column of similar polarity to that described above. Individual component identification was confirmed by mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC peak areas were converted to mg / 100 g using the 19:0 FAME internal injection standard prior to statistical analysis.

Statistical analyses

Fatty acid data were analysed for the fixed effects of sex, slaughter group/location, sire breed, SNP genotype and their second order interactions using both generalised (PROC GLM) and mixed (PROC MIXED) linear model procedures (SAS 2009) while the partial regressions of sire and herd were fitted as a random effects. Least square

means of fixed effects were obtained and tested for significance using the Tukey-Kramer adjustment test of paired values.

The full model was

$$Y_{ijklm} = G_i + SG_j + SB_k + SNP_l + (GSG)_{ij} + (GSB)_{ik} + (GSNP)_{il} + (SGSB)_{jk} + (SGSNP)_{jl} + (SBSNP)_{kl} + b_1(S - \overline{S})^2 + b_2(H - \overline{H})^2 + e_{ijklm}$$

where Y_{ijklm} is the $_{ijklm}$th observation of the dependent fatty acid with fixed effects of G_i of ith Gender (i=1,2), SG_j of jth slaughter group (j=1,2,3), SB_k of kth sire breed (k=1,2,3,4,5), SNP_l of lth SNP genotype (l=1,2), first order interaction effects $(GSG)_{ij}$, $(GSB)_{ik}$, $(GSNP)_{il}$, $(SGSB)_{jk}$, $(SGSNP)_{jl}$ and $(SBSNP)_{kl}$ of gender and slaughter group, gender and sire breed, gender and SNP genotype, slaughter group and sire breed, slaughter group and SNP genotype and sire breed and SNP genotype respectively. b_1 and b_2 are partial regression coefficients of sire and herd respectively, $b_1(S - \overline{S})^2$ and $b_1(H - \overline{H})^2$ fitted as random effects, and e_{ijklm} is a residual error term normally and independently distributed. All non-significant interactions were later removed from the final model.

Results

Pasture Fatty Acid Composition

The pasture fatty acid composition shows a trend closely related to available green feed offered to the flock (Figure 4.1). Sufficient green feed was available in October (ALA = 29% of measured fatty acids) through to December when weaning occurred. As green feed became limited with moisture stress, the percentage of ALA fell to 4.7% of measured fatty acids. In late January the flock had consumed the majority of available green feed and were relying on stubble residues, moisture stressed

irrigated pastures and grain supplementation. The two major fatty acids (16:0 and ALA) have comparable relative levels at the first sampling point, but ALA then decreases and 16:0 remains relatively stable. Table 4.1 summarises the total intramuscular fatty acid profiles for the entire flock and show overall that SFA and MUFA were the two dominant FA groups and high levels of variation were observed in all major fatty acids.

Figure 4.1 clearly shows a significant drop in all key PUFA over the experiment. The February sample shows the commencement of the decrease PUFA levels, in particular for ALA. Total SFA composition is the dominant fatty acid group at the end of the experiment consisting of 68% of all fatty acids. Irrigation water from February onwards was severely restricted and drought conditions were severe with only a very small green pick of pasture available. The impact on fatty acid composition is further evident in the March sample (Figure 4.1) showing low percentages of both MUFA and PUFA (8.9% MUFA and 13.4% PUFA). In April slaughter groups one and two and 40 animals to be used for a feeding trial were removed from the property decreasing grazing pressure on the pastures.

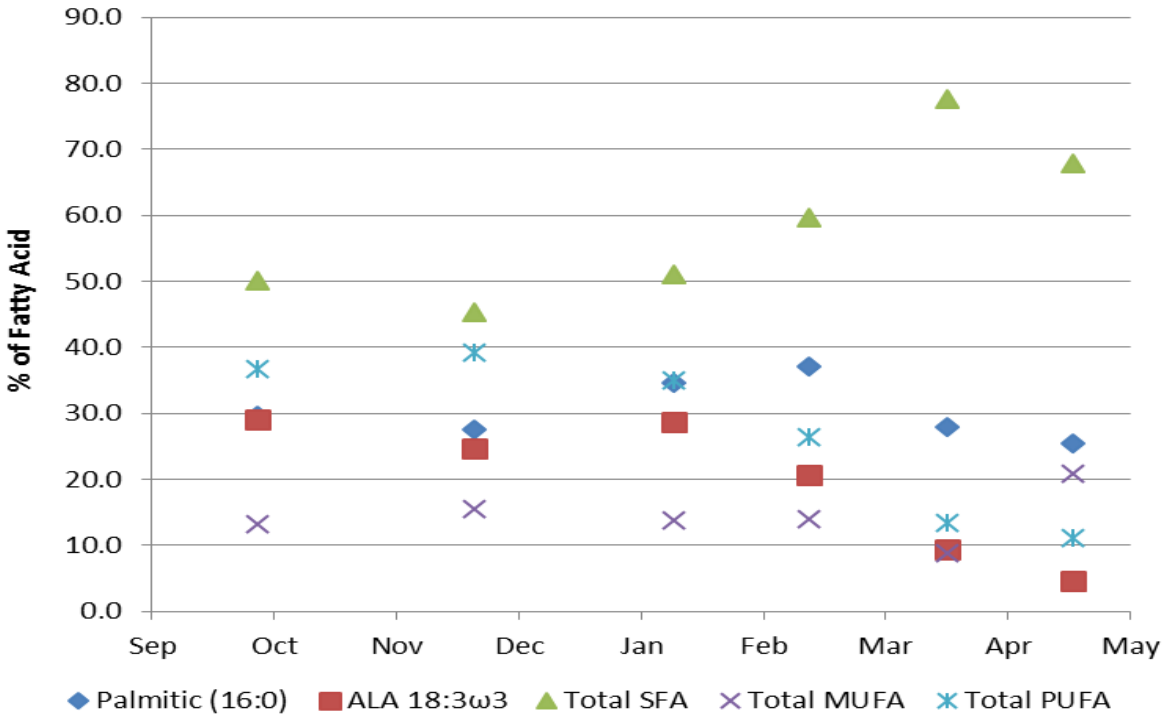

Figure 4.1 Pasture fatty acid profiles expressed as % of fatty acids measured. ALA and palmitic acid are initially the dominant fatty acids, however, over time ALA and overall PUFA decreases significantly and SFA becomes the predominant FA (n=365).

Growth

Growth rates of pasture based first cross Merino lambs were significantly ($P<0.05$) affected by the drought experienced in southern Tasmania during the first half of 2008. This is reflected in the depressed growth rates (Figure 4.2) where pasture quality declined from late March onwards as moisture stress reduced the pasture growth despite irrigation. The first slaughter group had the fastest growing animals in the flock, which were still grazing green pastures until slaughter and were a relatively small sample size (n=48). The second slaughter group comprised of animals grazing drought-stressed pasture with rapidly deteriorating nutrient quality as a result of the impact of temperature and moisture. Relocation of the remaining flock (n=365) to northern Tasmania, which was not in drought at the time, became necessary in May

2008. The negative effects of transportation and rumen adaptation to lush green feed was evident in the static period of growth till late June 2008 before live weight gain rose again (Figure 4.2).

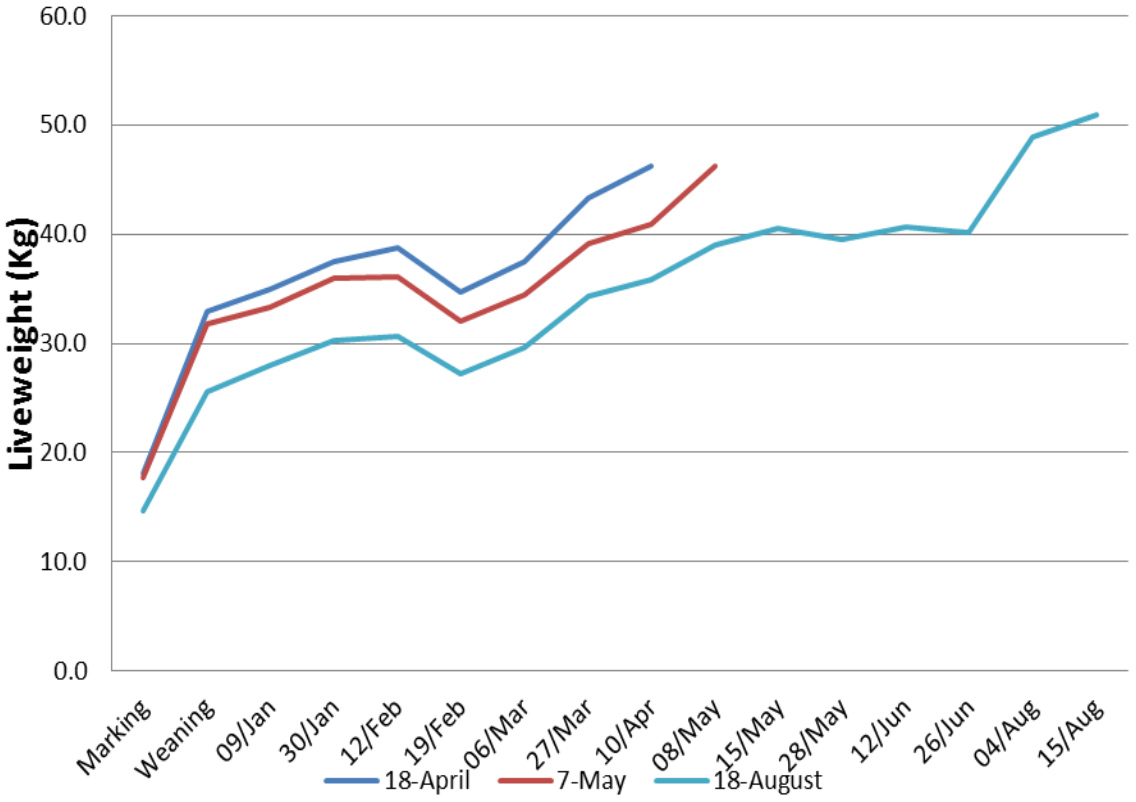

Figure 4.2 Mean live weights (kg) of pasture-based first cross Merino lambs from marking to slaughter in three slaughter groups (group 1, 18-April; group 2, 7-May; group 3, 18-August (n=365).

Slaughter Groups

Animals were divided into three slaughter groups, based on the attainment of the target slaughter weight of 44.5 kg. From Table 4.2 and Figure 4.2, it is evident that environmental conditions were the most significant factor affecting muscle fatty acid content in the lambs.

The first two slaughter groups were smaller sample sizes (n = 43 and 50, respectively), compared to the 203 animals in the third slaughter group. Muscle total

fatty acid contents for each of the three slaughter groups were; 1=1394 mg/100 g, 2=1335 mg/100 g, 3=1969 mg/100 g. The first two slaughter groups consisted of the top 20% liveweight of lambs at marking and the lambs continued to grow rapidly to reach the target slaughter weight of 44.5 kg. During the initial 3 months post weaning, all slaughter groups had comparable growth rates (Figure 4.2). Animals in the third slaughter group were initially lighter due to the prevailing drought conditions that necessitated their relocation. As a result, slaughter group 3 lambs took 122 days longer than their counterparts in slaughter group 1 to attain minimum slaughter weight.

There was a lower mean accumulation of intramuscular fatty acid content in slaughter groups 1 and 2 compared to slaughter group 3 suggesting that groups 1 and 2 favoured muscle growth over fatty acid storage and IMFC increased with time. Percentages of intramuscular fat were 2.5%, 2.3% and 3.5% for slaughter groups 1, 2 and 3, respectively (Table 4.3). Lambs in slaughter group 3 had 46% more intramuscular fat fatty acid content on average, compared to the first two slaughter groups.

As portrayed in Table 4.2, all but a few fatty acids were significantly affected ($P<0.05$) by slaughter date. Only 14:0, 20:3ω6 and 22:5ω6 were not significantly ($P<0.05$) affected by slaughter group as all the other fatty acids were highly significantly affected ($P<0.01$) by slaughter group (Table 4.2).

Sire Breed

Sire breed was significant ($P<0.05$) source of variation for the majority of SFA with Coopworth-sired lambs having higher level of SFA compared to their counterparts

from the other four sire breeds (Table 4.3). Lambs sired by the Coopworth breed also had a higher content of the LC omega-3 precursor ALA (24 mg/100 g). Despite the elevated ALA content, the mean EPA + DHA content of the Coopworth lambs was not significantly different ($P<0.05$) from the other sire breeds except for Texel. Texel sired animals had significantly ($P=0.045$) lower content of the LC omega-3 DHA compared to all other sire breeds.

Pasture-fed animals and fatty acid content

Overall, pasture-fed animals demonstrated highly varied content of each fatty acid analysed (Table 4.1), however, a greater biosynthesis and storage of intramuscular lipids was evident with older animals.

Figure 4.4 summarises the changes in the 3 major fatty acid groupings of SFA, MUFA and PUFA. SFA content steadily increased with time in East Friesian and Texel sired lambs. Conversely, lambs sired by Dorset and White Suffolk breeds demonstrated a decrease in SFA content at the second slaughter date. Coopworth sired lambs had the highest SFA content of 1125 mg/100 g among all sire breed groups which maximized during the first and second slaughters before it decreased to 1047 mg/100 g at the third slaughter date.

Mean MUFA content increased over time in Coopworth, East Friesian and Texel sire breeds. At the second slaughter date, Dorset and White Suffolk sired lambs had lower intramuscular contents than at the first kill before a gradual increase at the third slaughter date. Intramuscular PUFA content showed a steady accumulation with time in all sire breeds except White Suffolk. At the first slaughter date, White Suffolk intramuscular PUFA content was 132 mg/100 g and at the last slaughter

date, 4 months later, IM PUFA content increased to 143 mg/100 g showing significantly (P=0.048) lower PUFA accumulation to other sire breeds. White Suffolk PUFA content at the second slaughter date was less than observed for the first kill animals and dropped to 85 mg/100 g, however the difference was not significant.

Individual fatty acid profiles within slaughter groups

The difference in the content of individual fatty acids and slaughter date is shown in Table 4.3. During the 122 days which elapsed between the first and last slaughter groups, intramuscular fatty acid content increased by 46%. Slaughter groups 1 and 2 showed similar fatty acid contents, however, the diets were of declining nutritional value for the 2nd slaughter group and barley intake was elevated. This is evident with a number of individual fatty acids such as 14:0, 15:1ω6c, 16:0, 16:1, 17:0, 18:3ω3, 18:1ω9c, 18:1ω7c, 18:1ω7t, 18:1ω5c, 18:0, 20:0, 24:0, total SFA and total MUFA were lower in content than in the initial slaughter group. However a number of fatty acids did show continual increase in content over time: 17:1, 18:3ω6, 18:2ω6, 20:5ω3, 20:3ω6, 22:6ω3, 22:5ω3, 22:0 and total PUFA.

The ω6/ω3 ratio for the first two slaughter groups were 2.4:1 and 2.7:1 and notably dropped to 1.4:1 in the third slaughter group (Table 4.3). Intramuscular ALA content steadily increased with age in the Coopworth, East Friesian and Texel sired lambs. However, lambs sired by Dorset and White Suffolk demonstrated a decrease in ALA content at the second slaughter date. The mean content of LC omega-3 (EPA, DPA (3), DHA), biosynthesised from ALA, did show a continuous increase with time regardless of sire breed.

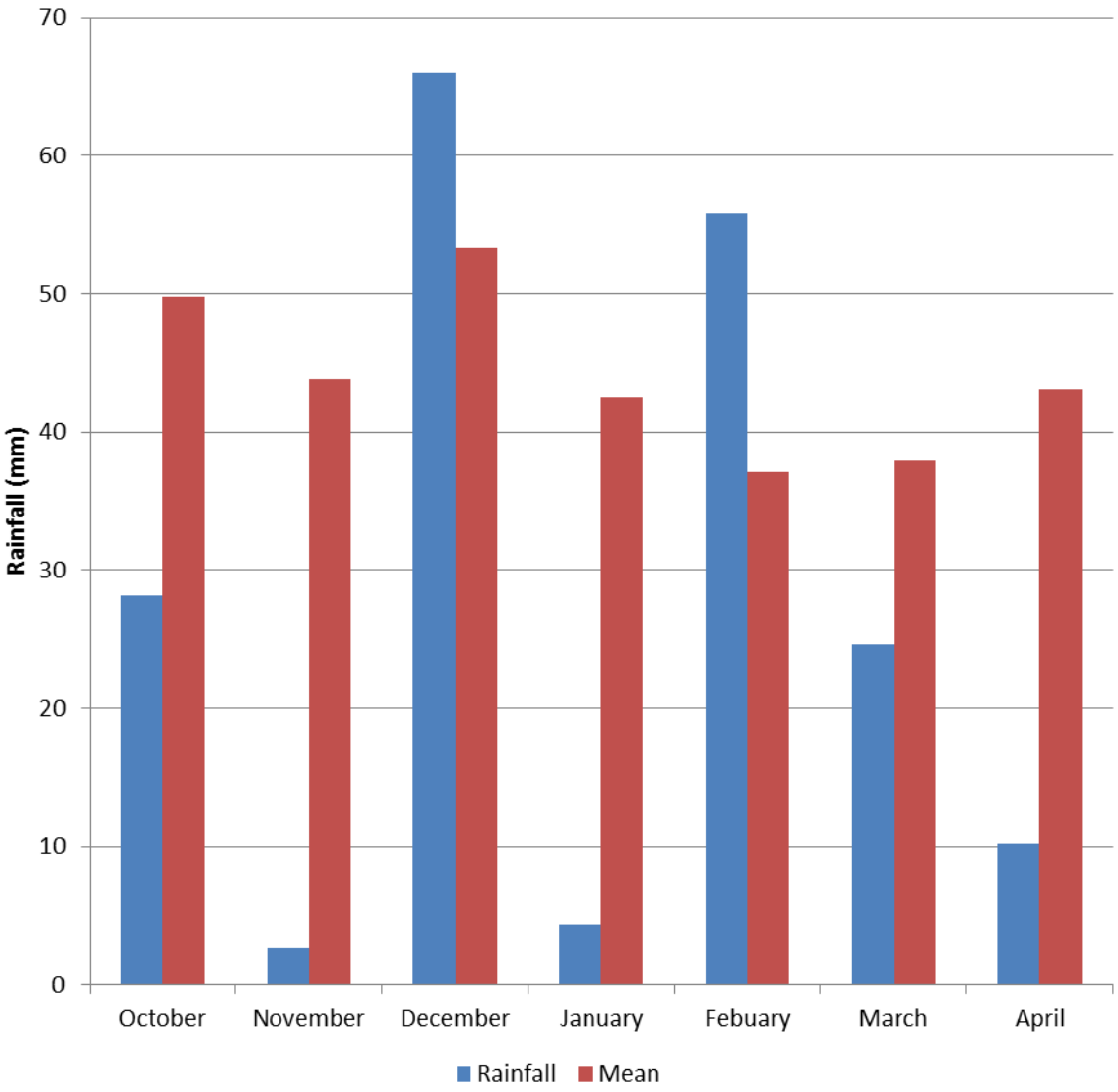

Figure 4.3 Actual rainfall and historical mean rainfall for the duration of the drought affected pastures at Lowlands weather station located 0.8km from the experimental site. The rainfall trends show below average rainfall for the majority of months with some isolated high rainfall events in December and February, however, total rainfall was 38% below average rainfall for the period. Source: Bureau of Meteorology Climate Information (B.O.M., 2013).

Table 4.1 Summary statistics of individual fatty acids (mg/100 g muscle) in first cross Merino sheep(n=364).

	Mean	Std Dev	Minimum	Maximum	Coefficient of variation	Variance
14:0	32.7	26	1	192	79.4	674.5
15:1ω6c	2.8	11	0	111	390.6	121.7
15:0	8.3	6.9	0	51	82.7	47.4
16:1	29.2	19.8	2	122	67.8	391.1
16:0	388.8	223.2	63	1374	57.4	49819.8
17:1	34.7	15.4	2	104	44.3	236.6
17:0	29.4	17.1	3	113	58.1	291
18:3ω6 GLA	0.4	0.6	0	4	145.9	0.4
18:4ω3 SDA	2.0	2.3	0	21	119.5	5.5
18:3ω3 ALA	26.1	15.3	0	104	58.5	233.8
18:2ω6 LA	65.9	29.9	12	244	45.4	893
18:1ω9c OA	667.9	390.5	5	2879	58.5	152452.8
18:1ω7c	29.2	18.1	6	139	62	327.7
18:1ω7t	60.9	50.6	0	369	83.1	2557.6
18:1ω5c	8.3	5.1	1	35	61.8	26.1
18:0	373.8	220.1	0	1549	58.9	48446.6
20:5ω3 EPA	10.1	5.4	1	33	53.2	28.8
20:4ω6 ARA	20.6	9.7	4	68	47.2	94.4
20:3ω6	2.9	1.5	0	7	50	2.1
20:4ω3 ETA	0.3	0.8	0	8	242.8	0.7
20:1ω9,11c	1.7	1.4	0	11	85.2	2
20:1ω7c	0.2	0.4	0	2	209.1	0.2
20:0	3.4	2	0	12	59.8	4
21:5ω3	0.1	0.3	0	2	321.9	0.1
22:5ω6 DPA6	0.1	0.5	0	4	448.1	0.3
22:5ω3 DPA3	9.8	5	0	34	50.4	24.5
22:6ω3 DHA	3.2	2	0	14	60.8	3.9
22:0	1.6	1.2	0	13	74.3	1.4
24:0	1.7	1.0	0	7.0	58.8	1.0
Other SFA	1.6	3.25	0	48	208.4	15.85
Other MFA	3.08	2.39	0.00	18.44	320.00	22.66
Other PUFA	0.26	0.44	0.00	3.60	438.60	0.24
EPA + DHA	13.3	7.0	1.0	45.0	53.0	49.5
Total SFA	844.2	487.2	72	3231	57.7	237326.6
Total MUFA	858	493.8	28	3380	57.6	243878.1
Total PUFA	142.8	62.7	29	534	43.9	3930.5
Total Omega 3	51.7	26.8	6.0	208	51.8	717.4
Omega 6 : Omega 3	2.0	0.8	0.7	6.0	38.7	0.6

SFA=Saturated fatty acids, MUFA=Monounsaturated fatty acids, PUFA=Polyunsaturated fatty acids, ALA=alpha linolenic acid, ARA= Arachidonic acid, EPA=Eicosapentaenoic acid, ETA= Eicosatetraenoic acid, DPA=Docosapentaenoic Acid, DHA=Docosahexaenoic acid, LA=Linoleic acid, OA= Oleic Acid, SDA=Stearidonic acid.

Table 4.2 Test of significance (P-values) for factors influencing Longissimus dorsi muscle fatty acid content of first cross Merino sheep (n=364).

Fatty acid	Sex	Slaughter Group	Sire Breed	FADS2 SNP	FABP4 SNP
14:0	0.7158	0.1587	**0.0161***	0.516	0.1216
15:1ω6c	0.8081	**0.0054*****	0.824	0.8022	0.4782
15:0	0.9448	**0.0016*****	**0.0112***	0.5245	0.0854
16:1	0.5304	**0.0135***	0.2132	0.7489	0.6884
16:0	0.6715	**0.0003*****	**0.0073****	0.7944	0.4167
17:1	0.9383	**0.0001*****	0.1948	0.5245	0.4106
17:0	0.9016	**0.0001*****	**0.0138***	0.6323	0.3189
18:3ω6 GLA	0.6702	**0.0001*****	0.3931	0.7023	0.5121
18:4ω3 SDA	**0.0463***	**0.0001*****	0.2106	0.497	**0.0158***
18:3ω3 ALA	0.9226	**0.0001*****	0.2847	0.7537	0.9778
18:2ω6	0.8153	**0.0001*****	0.0938	0.5962	0.3265
18:1ω9c OA	0.9315	**0.0001*****	0.1965	0.2186	0.6994
18:1ω7c	0.6741	**0.0001*****	0.3129	0.5082	0.7435
18:1ω7t	0.9138	**0.0001*****	**0.0098****	0.2264	0.7057
18:1ω5c	0.8558	**0.0001*****	**0.0046****	0.8394	0.9099
18:0	0.7698	**0.0002*****	**0.0029****	0.8334	0.2791
20:5ω3 EPA	0.4072	**0.0001*****	0.3074	0.0548*	0.4454
20:4ω6 ARA	0.4869	**0.0001*****	0.321	0.8107	0.0561
20:3ω6	0.9449	0.3753	0.7175	0.5049	0.837
20:4ω3 ETA	0.4452	**0.0306***	0.5337	0.8304	0.1401
20:1ω9,11c	0.3017	**0.0001*****	0.6689	0.3007	0.3805
20:1ω7c	0.7417	**0.0098*****	0.2587	0.3456	0.3973
20:0	0.7934	**0.0055*****	**0.0499***	0.6061	0.6454
22:6ω3 DHA	0.1917	**0.0001*****	**0.0449***	0.0499*	0.4386
22:5ω3 DPA	0.3453	**0.0001*****	0.653	0.8015	0.3168
22:5ω6	0.0548	0.9331	0.7597	0.7909	0.5153
22:0	0.5749	**0.0006*****	**0.006****	0.4468	0.1476
24:0	0.6334	**0.0017*****	0.1237	0.1846	0.3904
EPA + DHA	0.2876	**0.0001*****	0.2206	0.9592	0.302
Total SFA	0.9316	**0.0003*****	**0.0055****	0.7858	0.3162
Total MUFA	0.9435	**0.0001*****	0.1431	0.2359	0.6898
Total PUFA	0.6267	**0.0001*****	0.2283	0.6971	0.3037
Total Omega 3	0.5897	**0.0001*****	0.4779	0.8381	0.6609
Omega 6 : Omega 3	0.124	**0.0001*****	0.7523	0.8887	0.328

*P<0.05, **P<0.01, ***P<0.001, Abbreviations are as defined in Table 4.1.

Table 4.3 Least squares means ±S.E. of Longissimus dorsi muscle fatty acid content (mg/100 g) of first cross Merino sheep adjusted for sex, slaughter group and sire breed effects. Number of animals per group is denoted in brackets.

	Sex		Slaughter Group#			Sire Breed				
Fatty acid	*Male (135)*	*Female (200)*	*1(43)*	*2 (50)*	*3 (203)*	*CW (50)*	*EF (52)*	*DO (88)*	*TX (69)*	*WS (76)*
14:0	28.62 ± 2.2	29.66 ± 2.1	29.57 ± 5.9	21.00 ± 2.6	28.28 ± 1.7	40.47 ± 3.8	27.67 ± 3.8	25.86 ± 2.8	27.56 ± 2.7	24.13 ± 0.4
15:1ω6c	0.26 ± 0.8	0.58 ± 1.1	12.31 ± 0.0	11.60 ± 0.0	8.78 ± 0.9	1.50 ± 1.9	1.06 ± 2.2	0.74 ± 1.0	-0.21 ± 1.1	-0.98 ± 0.7
15:0	10.11 ± 0.6	10.06 ± 0.6	23.32 ± 1.6	22.11 ± 1.3	29.42 ± 0.3	12.99 ± 1	8.98 ± 0.7	9.22 ± 0.8	10.41 ± 0.9	8.85 ± 0.0
16:1	27.47 ± 1.6	28.89 ± 1.8	23.32 ± 2.9	22.11 ± 1.9	29.42 ± 1.5	31.39 ± 3	27.98 ± 3.4	31.40 ± 2.1	25.10 ± 1.7	25.01 ± 20.3
16:0	366.73 ± 17.4	377.27 ± 20.2	301.23 ± 35.1	283.35 ± 23.1	400.80 ± 16.0	461.95 ± 32.7	348.93 ± 35.2	395.80 ± 23.5	335.16 ± 21.4	318.18 ± 1.3
17:1	28.52 ± 1.2	28.38 ± 1.3	32.83 ± 2.7	34.99 ± 2.1	31.06 ± 1.1	33.10 ± 2.5	26.66 ± 2.2	28.99 ± 1.6	26.79 ± 1.8	26.69 ± 1.7
17:0	31.50 ± 1.4	31.25 ± 1.5	21.82 ± 2.8	20.08 ± 1.9	27.83 ± 1.2	38.92 ± 2.9	27.35 ± 2.4	31.36 ± 1.9	30.52 ± 0.1	28.71 ± 0.1
18:3ω6 GLA	0.60 ± 0.1	0.57 ± 0.1	0.32 ± 0.1	0.73 ± 0.1	0.30 ± 0.1	0.68 ± 0.1	0.70 ± 0.1	0.54 ± 0.1	0.48 ± 0.2	0.52 ± 0.2
18:3ω3 ALA	19.91 ± 1.2	19.76 ± 1.4	14.88 ± 1.5	13.27 ± 1.1	30.13 ± 1.1	23.97 ± 2.6	18.98 ± 1.9	18.44 ± 1.4	19.20 ± 2.0	18.58 ± 1.4
18:2ω6 LA	69.76 ± 2.3	70.60 ± 2.8	47.24 ± 4.2	52.24 ± 3.3	61.48 ± 2.2	81.41 ± 6	68.98 ± 4.1	67.66 ± 3.1	68.54 ± 38.9	64.29 ± 38.4
18:1ω9c OA	610.23 ± 29.9	606.43 ± 35.8	503.06 ± 53.6	498.40 ± 40.4	724.58 ± 28.2	723.16 ± 54.5	589.37 ± 68.1	607.99 ± 37.1	540.44 ± 1.9	580.69 ± 1.5
18:1ω7c	38.23 ± 1.6	37.34 ± 1.3	24.74 ± 3.5	23.42 ± 1.5	34.30 ± 1.2	40.90 ± 2.7	39.64 ± 3.6	38.47 ± 2.4	36.20 ± 5.9	33.71 ± 4.6
18:1ω7t	63.03 ± 3.8	62.42 ± 4.7	34.05 ± 5.0	29.79 ± 3.7	74.80 ± 3.7	84.34 ± 9.2	53.06 ± 6.2	64.99 ± 5.3	59.22 ± 0.5	52.02 ± 0.5
18:1ω5c	7.18 ± 0.4	7.29 ± 0.5	6.00 ± 0.7	5.07 ± 0.5	8.78 ± 0.4	9.74 ± 0.9	6.25 ± 0.5	6.86 ± 0.5	6.72 ± 0.4	6.60 ± 0.4
18:0	365.00 ± 17.0	357.77 ± 20.3	300.67 ± 35.8	272.82 ± 22.8	402.01 ± 15.5	473.09 ± 36.1	320.08 ± 1.4	354.31 ± 21.5	337.99 ± 1.1	321.44 ± 1.0
20:5ω3 EPA	8.54 ± 0.4	8.99 ± 0.5	5.29 ± 0.5	5.80 ± 0.4	11.52 ± 0.4	9.22 ± 0.8	8.86 ± 0.8	9.19 ± 0.5	7.54 ± 0.6	9.01 ± 0.6
20:4ω6 ARA	20.95 ± 0.8	21.74 ± 0.9	14.27 ± 1.3	16.29 ± 1.0	22.19 ± 0.7	23.17 ± 1.7	21.88 ± 0.2	22.03 ± 1.1	19.50 ± 0.2	20.15 ± 0.2
20:3ω6	2.80 ± 0.1	2.86 ± 0.1	2.12 ± 0.2	2.34 ± 0.2	2.68 ± 0.1	2.59 ± 0.2	2.75 ± 0.1	2.94 ± 0.1	2.70 ± 0.1	3.18 ± 0.1
20:1ω9,11c	2.36 ± 0.1	2.54 ± 0.1	1.39 ± 0.2	1.70 ± 0.2	1.92 ± 0.1	2.62 ± 0.2	2.39 ± 0.1	2.61 ± 0.2	2.30 ± 0.0	2.31 ± 0.0
20:0	3.49 ± 0.2	3.55 ± 0.2	3.04 ± 0.4	2.87 ± 0.2	3.30 ± 0.1	4.28 ± 0.3	3.07 ± 0.1	3.46 ± 0.0	3.50 ± 0.0	3.28 ± 0.0
22:6ω3 DHA	2.94 ± 0.2	3.23 ± 0.2	2.00 ± 0.3	2.03 ± 0.2	3.61 ± 0.1	3.07 ± 0.3	3.60 ± 0.3	3.15 ± 0.2	2.49 ± 0.2	3.11 ± 0.2
22:5ω6	0.00 ± 0.0	0.12 ± 0.1	0.05 ± 0.1	0.08 ± 0.0	0.08 ± 0.0	0.05 ± 0.1	0.09 ± 0.1	0.09 ± 0.1	-0.02 ± 0.1	0.10 ± 0.1
22:5ω3 DPA	9.00 ± 0.4	9.50 ± 0.5	6.10 ± 0.5	7.25 ± 0.5	11.60 ± 0.3	9.53 ± 0.8	9.41 ± 0.7	9.79 ± 0.5	8.62 ± 0.6	8.93 ± 0.5
22:0	1.54 ± 0.1	1.48 ± 0.1	1.11 ± 0.3	1.23 ± 0.1	1.63 ± 0.1	1.94 ± 0.3	1.36 ± 0.1	1.51 ± 0.0	1.51 ± 0.0	1.25 ± 0.0
24:0	1.72 ± 0.1	1.77 ± 0.1	1.61 ± 0.1	1.58 ± 0.1	2.01 ± 0.1	2.08 ± 0.2	1.62 ± 0.1	1.73 ± 0.1	1.64 ± 0.1	1.65 ± 0.1
EPA+DHA	13.28 ± 0.6	14.08 ± 0.6	7.19 ± 0.8	7.84 ± 0.6	15.21 ± 0.5	14.28 ± 1.2	13.91 ± 1.0	14.26 ± 0.8	11.84 ± 0.8	14.12 ± 0.8
EPA+DPA+DHA	20.69 ± 0.9	21.94 ± 1.1	13.49 ± 1.2	15.27 ± 1.0	26.93 ± 0.8	21.99 ± 1.7	22.04 ± 1.6	22.21 ± 1.1	18.85 ± 1.3	21.49 ± 1.2
Total SFA	809.30 ± 37.9	813.98 ± 44.2	670.00 ± 79.9	613.25 ± 50.3	880.13 ± 34.5	1036.86 ± 75.5	738.90 ± 71.2	825.89 ± 49.6	749.52 ± 49.9	707.05 ± 45.5
Total MUFA	797.52 ± 37.8	793.60 ± 45.3	631.73 ± 65.8	621.48 ± 49.4	941.14 ± 35.6	950.05 ± 71.1	765.42 ± 82.5	802.13 ± 47.5	716.52 ± 50.1	743.67 ± 47.7
Total PUFA	137.91 ± 4.8	141.36 ± 5.8	93.12 ± 7.4	100.57 ± 6.1	147.77 ± 4.5	158.03 ± 11.5	138.71 ± 8.1	137.69 ± 6.0	132.31 ± 7.8	131.42 ± 5.8
Total Omega 3	41.11 ± 2.0	42.58 ± 2.5	28.01 ± 2.5	28.05 ± 1.9	59.87 ± 1.8	47.18 ± 4.5	41.43 ± 3.4	41.51 ± 2.5	38.50 ± 3.2	40.59 ± 2.6
ω6 : ω3 Ratio	2.52 ± 0.1	2.40 ± 0.1	2.38 ± 0.1	2.70 ± 0.1	1.37 ± 0.0	2.54 ± 0.1	2.42 ± 0.1	2.47 ± 0.1	2.50 ± 0.1	2.38 ± 0.1
Total IMF %	3.0 ± 0.1	3.0 ± 0.1	2.5 ± 0.1	2.3 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	3.1 ± 0.1

 # Slaughter Group 1=18/4/2008 fastest growth, 2=7/5/2008 moderate growth and 3=18/8/2008 slowest growth, Sire breeds CW=Coopworth, EF=East Friesian, DO=Dorset, TX=Texel, WS=White Suffolk, Total IMF % = total intramuscular fatty acid percentage. Abbreviations are as defined in Table 4.1.

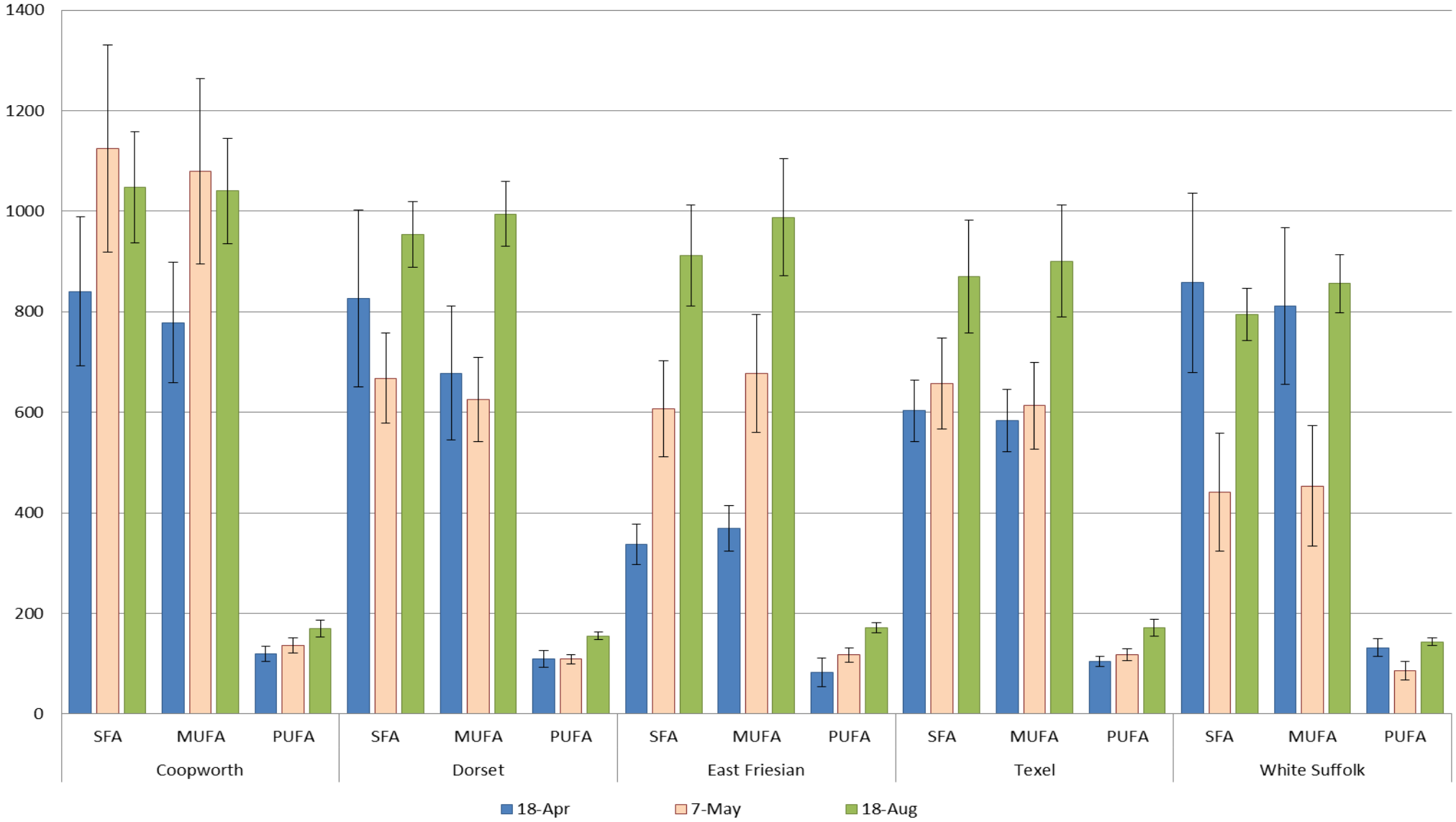

Figure 4.4 Mean total intramuscular content (mg/100 g of muscle) of saturated, monounsaturated and polyunsaturated fatty acid groups for each sire breed at three different slaughter dates (group 1, 18-April; group 2, 7-May; group 3, 18-August).

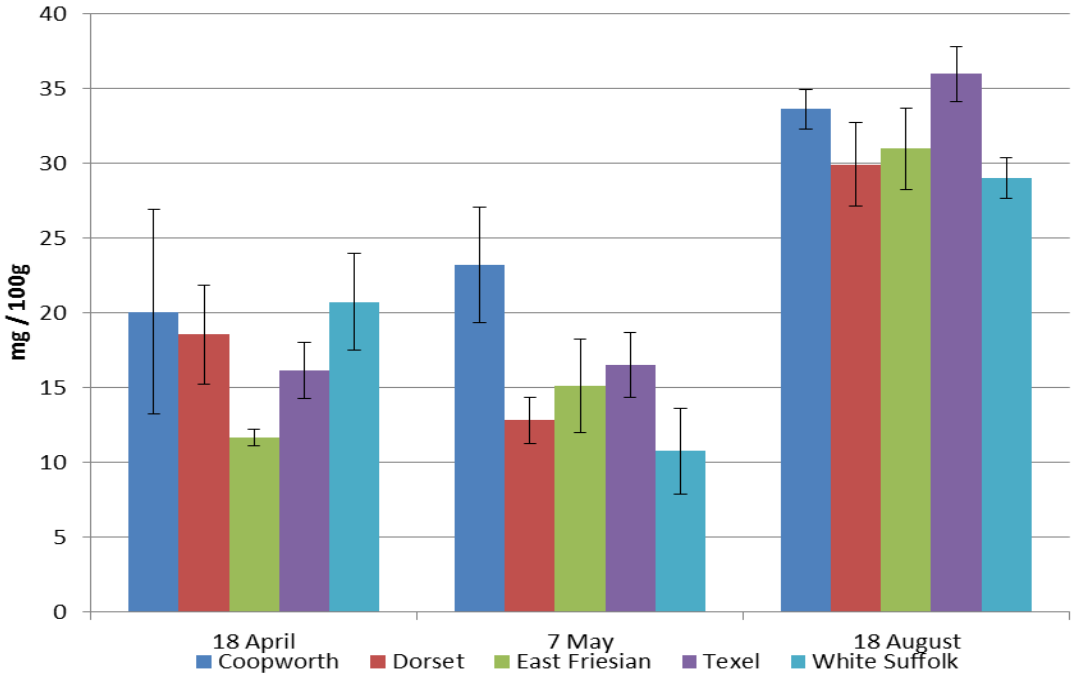

Figure 4.5 Intramuscular ALA content (mg/100 g of muscle) by breed and slaughter date (n=346).

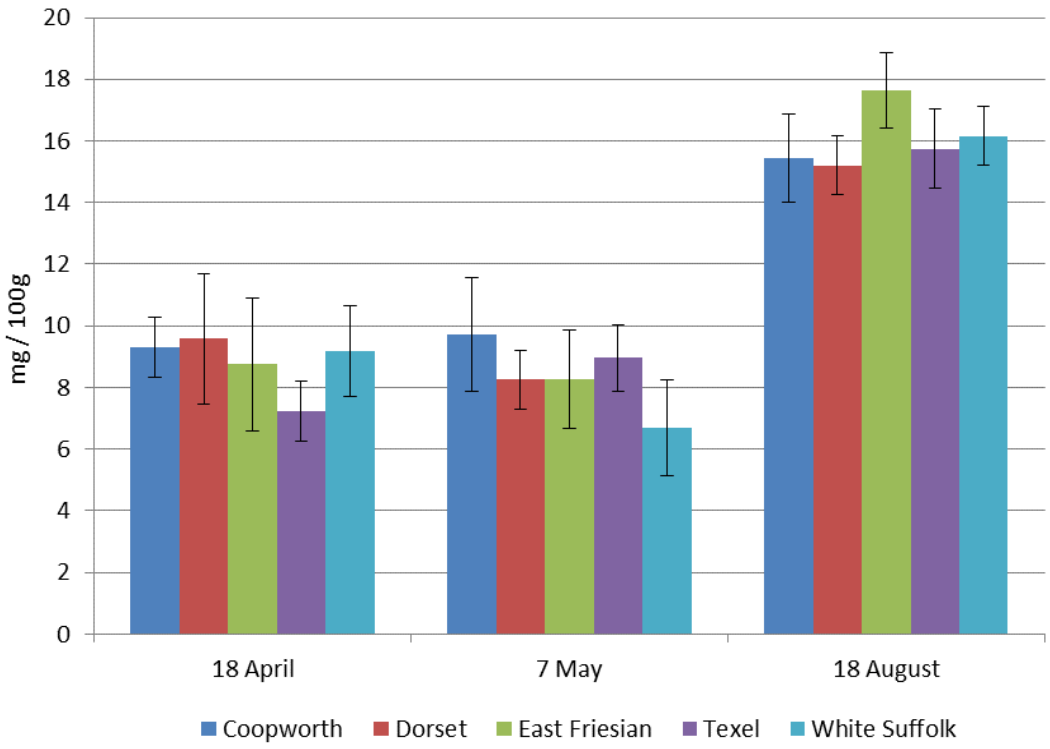

Figure 4.6 Intramuscular EPA content (mg/100 g of muscle) by breed and slaughter date (n=346).

Discussion

Pasture Fatty Acid Profiles

The percentages of measured fatty acids in the spring and early summer pasture samples are consistent with levels observed in previous non-stressed perennial ryegrass dominant pastures with ALA and palmitic acid as the major fatty acids (Demirel *et al.*, 2006; Gallardo *et al.*, 2011). These results differ from profiles observed for glasshouse trials which have shown ALA as the dominant FA consisting of up to 75.5% of measured fatty acids and palmitic acid at lower composition (Boufaied *et al.*, 2003; Clapham *et al.*, 2005; Gilliland *et al.*, 2002).

Pasture fatty acid profiles showed a switch in dominance of FA groups as summer and drought progressed with an initial trend of equal PUFA and SFA dominance, however, PUFA then declined and SFA became the dominant fatty acid group (Figure 4.1). The decline in PUFA is most likely occurring due to moisture stress and limited growth, however, other factors such as flowering, plant maturity and grazing intensity have a negative effect on PUFA and FA content (Boufaied *et al.*, 2003; Dewhurst *et al.*, 2001; Elgersma *et al.*, 2003). Time point sampling experiments over summer with pastures being grazed or conserved have reported that stem and dry matter increases in the sward samples with time and an overall decline in PUFA content of up to 35% occurs, so some decline in PUFA content would be expected (Clapham *et al.*, 2005; Demirel *et al.*, 2006; Dewhurst *et al.*, 2001). ALA content

decreased by 84% over the experiment suggesting factors outside of grazing, maturity and time of sampling were influencing the PUFA content. This is most likely moisture stress/drought and results in very limited green material being available to graze. The entire region of SE Tasmania was experiencing below average rainfall and high evaporation rates making this season atypical and most likely the most significant factor driving the decline in PUFA composition of the pasture (Figure 4.3). Two large rainfall events occurred in December and early February giving pastures a chance to produce lush green growth. Pasture was not sampled in December, although a small increase in PUFA composition is noted for the 11th of January sample period (Figure 4.1) before a continual decline in PUFA content.

The fields in the February sample were rested for the six weeks prior to sampling and irrigated; in addition three 14+ mm rainfall events occurred for the month before the animals were moved back for grazing. The February sample date coincided with the first day that the pastures were grazed with (2.4 t/Ha Dry Matter) and were green and actively growing. Dewhurst (2000) reported an increase of PUFA during vegetative re-growth post grazing or harvesting however these samples did not replicate this response and an increase in SFA was observed and PUFA continually decreased.

Growth Rates

Mean average daily weight gain for lambs from weaning till 7/05/2008 across the three slaughter groups were comparable with an average ADG of 122±31 g / day, however, liveweight differed greatly. These ADG are lower than those reported in ryegrass dominant pasture experiments where gains ranging from 170 g / day to over 220 g / day are recorded (Annett *et al.*, 2011; Carson *et al.*, 2001; Demirel *et al.*,

2006; Gallardo *et al.*, 2011). These results indicate clearly that the flock was not performing to its full potential for liveweight gain.

The animals were joined as per Australian best practice of allowing two parturition cycles of the ewes to maximise conception rates and control the period of lambing to a six week window (Jolly and Wallace, 2007). Animals in slaughter group three were 17% lighter than the other two slaughter groups at weaning and this may be explained by birth date as the flock had a mean ADG of 184 g / day for this period so animals born in week 1 could have grown an extra 6.44 kg to lambs born in week six. Therefore, a combination of environmental and ewe nutrition factors would have been influential in contributing to the observed genetic variation in weight at weaning and thereafter which is in agreement with the observations reported by (Duddy *et al.*, 2005).

Lambs in slaughter groups 1 and 2 belonged to the top 20% of the heaviest animals at weaning, and they maintained this growth advantage by attaining slaughter weight earlier than their group 3 counterparts. In general, the entire flock showed reasonable growth rates when pasture was available. However, the significantly depressed period of growth immediately after relocation was expected, but not for as long as was experienced. It is generally accepted that ruminants relocated to a significantly different feed source will need a period of rumen biota adaptation (Duddy *et al.*, 2005). Within 21 days on the average, the rumen is expected to adapt and live weight gain is anticipated to increase assuming ample feed is available. In this study, the animals remained in a static state for nearly 6 weeks before live weight gain resumed. The flock was consuming specialty grass fodder crops and had ample feed on offer, but the derived nutrients were apparently being channelled towards maintenance rather than growth. The weather for the months of May and

June was particularly inclement with waterlogged soils, snow falls, severe frost and rain which was in stark contrast to southern Tasmania. As a result, the sheep would have had increased metabolic requirements to meet maintenance, and coupled with the rumen adaptation occurring following relocation and the stress of transportation would have meant it was difficult for animals to take in enough nutrients to meet the energy and protein requirements beyond maintenance.

Sex Effect

The effect of sex on the intramuscular PUFA content was only statistically significant for 18:4ω3 (SDA) which is a minor fatty acid with a content of 2 mg/100 g. The apparent lack of effect due to sex is contrary to studies in humans where females were shown to have a marginally more efficient ALA to EPA conversion pathway (Burdge, 2004).

Kitessa et al., (2010) investigated LC omega-3 content (EPA + DHA) in lambs under different finishing conditions and also found sex was not a significant source of variation. Apart from the obvious species differences between ruminants in this study and monogastrics in the study by Burge (2003), this investigation utilised intramuscular fatty acid profiles as opposed to whole lipid pool. This result has a positive bearing for healthier elevated LC omega-3 content in lamb meat marketing as retailers do not have to rely on knowing the sex of the carcass to ensure elevated levels of LC omega-3. This is also good for producers as it means both wethers and ewes reared on pasture can be marketed for potential price premiums.

Slaughter Group

The results clearly demonstrate the effects that quality and quantity of green grass can have on muscle fatty acid profiles, particularly LC omega-3. Results for the first

two slaughter batches indicate that drought was having a negative impact on the flock PUFA content and analysis of pasture samples confirm the decreased intakes of ALA (Figure 4.1). Under normal conditions, weaner lambs require a protein-rich feed intake of at least 3% of their liveweight as they are rapidly depositing very lean tissue and skeleton until reaching approximately 34 kg. After reaching 34 kg liveweight, the muscle and skeletal maturation is nearly complete and feed intake decreases and fat deposition increases (Duddy *et al.*, 2005). Given the rapid growth rates of animals in slaughter groups 1 and 2, the period where fat deposition was expected to surpass protein accretion was limited and with almost nil green grass, it is not surprising that the percentages of IMF was so low.

Intramuscular LC omega-3, specifically EPA + DHA, was low in the first two slaughter groups in comparison with slaughter group 3 (7 mg/100 g, 7 mg/100 g and 15 mg/100 g, respectively). The contents of EPA + DHA in slaughter groups 1 and 2 animals were markedly lower than values recorded in recent Australian trials (Mortimer *et al.*, 2010; Ponnampalam *et al.*, 2010).The samples used for these other Australian trials were from the Information Nucleus Flock (INF) as part of the Cooperative Research Centre for Sheep Industry Innovation (Sheep CRC) scientific program. The animals were raised across seven sites representative of major sheep production regions across Australia and the authors found that site was affecting EPA + DHA concentrations, which was related to available green grass at each site; animals did meet “source” levels based on a 135 g wet muscle serving at a number of locations (Pannier *et al.*, 2010).

Kitessa & Liu et al., (2010) in describing a subset of Sheep CRC INF, concluded that pasture reared animals had greater LC omega-3 content than concentrate fed animals and that the greenness of pasture was an important factor contributing to LC

omega-3 content. This is in agreement with findings from other ruminant pasture based trials (Clapham *et al.*, 2005; Cosgrove *et al.*, 2004; Dierking, 2008; Howe *et al.*, 2006; Ponnampalam *et al.*, 2009; Aurousseau *et al.*, 2007e). In pastures, there is biochemical evidence that chlorophyll - the green pigment in grass - is directly related to ALA content, further strengthening the green grass observations and need for the availability of non-stressed pastures to maximise ALA intake (Clapham *et al.*, 2005; Dierking, 2008; Dierking *et al.*, 2010; Dewhurst *et al.*, 2002).

Abiotic pasture stress and lower ALA content in the pasture and animal muscle due to drought conditions was reflected by a decrease in intramuscular ALA content in the first two slaughter groups (Figures 4.1 and 4.4). In contrast, animals in the third slaughter group were relocated to greener pastures and contained double the ALA IMF content suggesting the pastures contained higher levels of ALA compared to the initial drought-affected pastures. Figure 4.6 shows that EPA contents were highly varied by sire breed, supporting other studies illustrating variation was as great within breeds as across breeds (Cooper *et al.*, 2004; Mortimer *et al.*, 2010; Wachira *et al.*, 2002).

All the animals had *ad libitum* access to barley from feeders and crop stubbles for the duration of the experiment. However, the greatest consumption was in March and April when an excess of 1 tonne of feed per week was being consumed, highlighting the fact that the pastures were not meeting the nutrient requirements of the lambs. Slaughter group 3 lambs did not consume the entire 1.2 tonnes of barley on offer over the three months indicating pastures were meeting requirements. As a consequence of high grain intakes and rumen acidification, the biohydrogenation of ingested PUFA would also have occurred at a higher level in slaughter groups 1 and

2 lambs compared to those in slaughter group 3 ultimately leading to lower EPA + DHA content.

The ω6:ω3 ratios are well below the suggested dietary ratio of 5:1 being 2.4:1, 2.7:1 and 1.37:1 for each consecutive slaughter group respectively (Table 4.3). This trend is interesting because despite the relatively higher intake of the ω6 PUFA containing barley initially, it did not drive the ratio to greater than 5:1. This finding suggests that the lambs were still actively foraging for grass until relocation and were not relying entirely on grain to meet metabolic requirements. There is a big difference between sites though with the oldest slaughter group having a higher IMF content and a 1.37:1 ω6:ω3 ratio which shows that although drought effected pasture does decrease the nutritional benefits of lamb meat, it is still not outside reasonable healthy eating guidelines.

Conclusion

Drought has a negative effect on the ability of lamb to deposit high levels of healthy intramuscular fat with a desirable ω6:ω3 ratio and enhanced LC omega-3 content. Rearing lambs on pasture with ample green grass is a good indicator that there is abundant shorter-chain omega-3 - ALA - which ultimately leads to elevated levels of the LC omega-3 - EPA and DHA. Sex did not have a significant effect on intramuscular LC omega-3 content which is good for producers aiming for elevated LC omega-3 content. Relocation of the drought-affected flock did not result in rapid compensatory growth, however, the overall health-beneficial effects in terms of the meat fatty acid profiles, did improve. Sire breed significantly affected the SFA and some MUFA, but not LC omega-3 content. Large variation in fatty acid contents were

observed in all slaughter groups and further investigation into genetic parameters controlling LC omega-3 content is required to fully maximise potential gains from enhanced LC omega-3 containing lamb.

References

Annett R.W., Carson A.F., Dawson L.E.R., Kilpatrick D.J. (2011) Effects of dam breed and dietary source of n-3 polyunsaturated fatty acids on the growth and carcass characteristics of lambs sourced from hill sheep flocks. Animal 5:1023-1035. DOI: 10.1017/s1751731110002703.

Aurousseau B., Bauchart D., Faure X., Galot A.L., Prache S., Micol D., Priolo A. (2007) Indoor fattening of lambs raised on pasture: (1) Influence of stall finishing duration on lipid classes and fatty acids in the longissimus thoracis muscle. Meat Science 76:241-252. DOI: 10.1016/j.meatsci.2006.11.005.

B.O.M. (2013) Monthly rainfall Richmond (Lowlands) Bureau of Meterology.

Bauman D.E., Perfield II J.W., de Veth M.J., Lock A.L. (2003) New perspectives on lipid digestion and metabolism in ruminants, Proc. Cornell Nutr. Conf. pp. 175-189.

Bligh E.G., Dyer W.J. (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Boufaied H., Chouinard P.Y., Tremblay G.F., Petit H.V., Michaud R., Belanger G. (2003) Fatty acids in forages. I. Factors affecting concentrations. Canadian Journal of Animal Science 83:501-511.

Burdge G. (2004) alpha-linolenic acid metabolism in men and women: nutritional and biological implications. Current Opinion in Clinical Nutrition and Metabolic Care 7:137-144.

Caparra P., Foti F., Scerra M., Cilione C., Vottari G., Galofaro V., Sinatra M.C., Scerra V. (2007) Influence of feeding system on fatty acid composition of suckling lambs. Options Mediterraneennes. Serie A, Seminaires Mediterraneens:95-99.

Carson A.F., Irwin D., Kilpatrick D.J. (2001) A comparison of Scottish Blackface and Cheviot ewes and five sire breeds in terms of lamb output at weaning in hill sheep systems. Journal of Agricultural Science 137:221-233.

Clapham W.M., Foster J.G., Neel J.P.S., Fedders J.M. (2005) Fatty acid composition of traditional and novel forages. Journal of Agricultural and Food Chemistry 53:10068-10073. DOI: 10.1021/jf0517039.

Cooper S.L., Sinclair L.A., Wilkinson R.G., Hallett K.G., Enser M., Wood J.D. (2004) Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. Journal of Animal Science 82:1461-1470.

Cosgrove G.P., Anderson C.B., Knight T.W., Roberts N.J., Waghorn G.C. (2004) Forage lipid concentration, fatty acid profile and lamb productivity. Proceedings of the New Zealand Grassland Association 66:251-256.

Demirel G., Ozpinar H., Nazli B., Keser O. (2006) Fatty acids of lamb meat from two breeds fed different forage: concentrate ratio. Meat Science 72:229-235. DOI: 10.1016/j.meatsci.2005.07.006.

Dewhurst R.J., Moorby J.M., Scollan N.D., Tweed J.K.S., Humphreys M.O. (2002) Effects of a stay-green trait on the concentrations and stability of fatty acids in perennial ryegrass. Grass and Forage Science 57:360-366. DOI: 10.1046/j.1365-2494.2002.00336.x.

Dewhurst R.J., Scollan N.D., Youell S.J., Tweed J.K.S., Humphreys M.O. (2001) Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. Grass and Forage Science 56:68-74.

Dierking R.M. (2008) Fatty acid variation between forage species and within populations populations and fatty acid content of beef finished on pasture with different forrage species, Division of Plant Sciences, University of Missouri. pp. 84.

Dierking R.M., Kallenbach R.L., Roberts C.A. (2010) Fatty Acid Profiles of Orchardgrass, Tall Fescue, Perennial Ryegrass, and Alfalfa. Crop Science 50:391-402. DOI: 10.2135/cropsci2008.12.0741.

Droulez V., Williams P., Levy G., Stobaus T., Sinclair A.J. (2006) Composition of Australian red meat 2002 2. Fatty acid profile. Food Australia 58:335-341.

Duddy G., Hegarty R.S., White A. (2005) Managing for lamb production, in: D. Cottle (Ed.), Sheep Production, School of Rural Science and Agriculture University of New England, Armidale, Australia. pp. 9-1 - 9-20.

Elgersma A., Ellen G., Horst H.v.d., Muuse B.G., Boer H., Tamminga S. (2003) Influence of cultivar and cutting date on the fatty acid composition of perennial ryegrass (Lolium perenne L.). Grass and Forage Science 58:323-331. DOI: 10.1046/j.1365-2494.2003.00384.x.

FSANZ F.S.A.N.Z.-. (2003) Standard 1.2.8 Nutrition information requirements, Canberra , Australia.

Gallardo M.A., Pulido R., Gallo C. (2011) Fatty acid composition of Longissimus dorsi muscle of suffolk down lambs fed on different drlyand forages. Chilean Journal of Agricultural Research 71:566-571.

Gilliland T.J., Barrett P.D., Mann R.L., Agnew R.E., Fearon A.M. (2002) Canopy morphology and nutritional quality traits as potential grazing value indicators for Lolium perenne varieties. Journal of Agricultural Science 139:257-273. DOI: 10.1017/s0021859602002575.

Givens D.I., Khem K.E., Gibbs R.A. (2006) The role of meat as a source of n-3 polyunsaturated fatty acids in the human diet. Meat Science 74:209-218. DOI: 10.1016/j.meatsci.2006.04.008.

Howe P., Meyer B., Record S., Baghurst K. (2006) Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. Nutrition 22:47-53. DOI: 10.1016/j.nut.2005.05.009.

Jolly S., Wallace A. (2007) Best Practice for production feeding of lambs: a review of the litterature, in: B. Brice (Ed.), Meat and Livestock Australia, Sydney. pp. 179.

Kitessa S., Liu S.M., Briegel J., Pethick D., Gardner G., Ferguson M., Allingham P., Nattrass G., McDonagh M., Ponnampalam E., Hopkins D. (2010) Effects of intensive or pasture finishing in spring and linseed supplementation in autumn on the omega-3 content of lamb meat and its carcass distribution. Animal Production Science 50:130-137. DOI: 10.1071/an09095.

Kitessa S.M., Gulati S.K., Ashes J.R., Scott T.W., Fleck E. (2001) Effect of feeding tuna oil supplement protected against hydrogenation in the rumen on growth and n-3 fatty acid content of lamb fat and muscle. Australian Journal of Agricultural Research 52:433-437. DOI: 10.1071/ar00058.

McAfee A.J., McSorley E.M., Cuskelly G.J., Moss B.W., Wallace J.M.W., Bonham M.P., Fearon A.M. (2010) Red meat consumption: An overview of the risks and benefits. Meat Science 84:1-13. DOI: 10.1016/j.meatsci.2009.08.029.

MLA. (2011) Industry overview Meat and Livestock Australia.

Myers N., Kent J. (2003) New Consumers: The Influence of Affluence on the Environment. Proceedings of the National Academy of Sciences of the United States of America 100:4963-4968.

Nichols P.D., Petrie J., Surinder S. (2010) Long-chain omega-3 oils - an update on sustainable sources. Nutrients 2:572-585.

Noble R. (1981) Digestion, absorption and transport of lipids in ruminant animals., in: W. Christie (Ed.), Lipid Metabolism in Ruminant Animals, Pergamon Press., Oxford, UK. pp. 57-93.

Pannier L., Ponnampalam E.N., Gardner G.E., Hopkins D.L., Ball A.J., Jacob R.H., Pearce K.L., Pethick D.W. (2010) Prime Australian lamb supplies key nutrients for human health. Animal Production Science 50:1115-1122. DOI: 10.1071/an10132.

Ponnampalam E.N., Hopkins D.L., Butler K.L., Dunshea F.R., Sinclair A.J., Warner R.D. (2009) Polyunsaturated fats in meat from Merino, first- and second-cross sheep slaughtered as yearlings. Meat Science 83:314-319. DOI: 10.1016/j.meatsci.2009.05.018.

Ponnampalam E.N., Warner R.D., Kitessa S., McDonagh M.B., Pethick D.W., Allen D., Hopkins D.L. (2010) Influence of finishing systems and sampling site on fatty acid composition and retail shelf-life of lamb. Animal Production Science 50:775-781. DOI: 10.1071/an10025.

Scerra M., Caparra P., Foti F., Cilione C., Zappia G., Motta C., Scerra V. (2011) Intramuscular fatty acid composition of lambs fed diets containing alternative protein sources. Meat Science 87:229-233. DOI: 10.1016/j.meatsci.2010.10.015.

Skapetas B., Sinapis E., Katanos I., Laga V., Eleftheriadou A. (2009) Factors influencing fatty acid composition of lamb meat and possibilities for its improvement. Epitheorese Zootehnikes Epistemes:41-58.

Trautwein E.A. (2001) n-3 Fatty acids - physiological and technical aspects for their use in food. European Journal of Lipid Science and Technology 103:45-55. DOI: 10.1002/1438-9312(200101)103:1<45::aid-ejlt45>3.3.co;2-0.

Voicu I., Ropota M., Voicu D. (2010) Research on the influence of new diet formulations on the quality of fattening lambs meat. Archiva Zootechnica 13:47-52.

Wachira A.M., Sinclair L.A., Wilkinson R.G., Enser M., Wood J.D., Fisher A.V. (2002) Effects of dietary fat source and breed on the carcass composition, n-3 polyunsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. British Journal of Nutrition 88:697-709. DOI: 10.1079/bjn2002727.

Williams P., Droulez V. (2010) Australian red meat consumption - implications of changes over 20 years on nutrient composition. Food Australia 62:87-94.

Chapter 5

Long-chain omega-3 content in sheep *Longissimus dorsi* muscle is not associated with FADS2 and FABP4 single nucleotide polymorphisms

Abstract

The primary objective of this Chapter was to better understand the genetic variability in sheep muscle omega-3 long-chain (≥C_{20}) polyunsaturated fatty acid content (LC-PUFA, also termed LC omega-3) and relationships with lipid synthesis and fat metabolism-related genes. Therefore, this study investigated the association between polymorphisms of the fatty acid binding proteins (FABP) and Delta-6 desaturase (FADS2) gene clusters. Three putative single nucleotide polymorphisms (SNP) were genotyped in *Longissimus dorsi* muscle samples from 362 crossbred prime lambs sired by five genetically divergent rams. Total intramuscular LC omega-3 content was determined using gas chromatography. Mixed model statistical analyses revealed that none of the putative SNP was significantly ($P<0.05$) associated with intramuscular levels of eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic (DHA, 22:6 ω3) acids. Therefore, the potential as a molecular marker breeding tool to predict intramuscular EPA + DHA content and/or composition to improve genetic progress in sheep meat breeding programs seems limited. To date it appears that the availability of green grass for the duration of the animal's lifetime has the biggest impact on LC omega-3 content. This experiment demonstrated drought has a negative impact on LC omega-3 content and the variability of LC omega-3 content is more than likely explained by diet than genetic associations with polymorphism at FADS2 or FAPB4 gene regions.

Keywords Long-chain omega-3 fatty acids, FADS2, FABP1, FABP2, prime lambs, SNPs

Introduction

The competition to supply protein globally is increasing and livestock are a key source with chicken and pork dominating the market (ABARE, 2009). As a result, red meat has had to re-position itself from being seen as a cheap protein source in order to maintain and improve its market share over the last 20 years (Piggott et al., 1996; Holloway et al., 2000). Sheep meat and lamb in particular, over the last decade have become seen as a luxury item or more expensive meat for everyday consumption compared to chicken, pork or beef. The change in market behaviour has necessitated a need to develop points of difference for sheep meat to maintain market share (Williams and Droulez, 2010). Significant work has therefore been undertaken to improve sheep meat eating quality and lamb meat has become a significant component of the Australian sheep industry with a farm gate value that has in recent times increased from $0.5 to $2.2 billion (Rowe, 2010).

The fatty acid composition of adipose and muscle tissues of ruminants is an important meat eating quality trait because of its relationship with flavour and tenderness. Omega-3 long-chain (≥C_{20}) polyunsaturated fatty acids (LC-PUFA, also termed LC omega-3) are well documented for their beneficial effects on human health, for example reducing the risk of atherosclerosis and other heart-related complications and improving the levels of these key fatty acids in red meat is of great interest to Australian sheep meat producers (Givens *et al.*, 2006; Howe *et al.*, 2006). Sheep in Australia are typically grazed within extensive pasture based grazing

systems. The base feed, green grass, is a rich source of the shorter chain (C_{18}) omega-3 fatty α-linolenic acid (ALA, 18:3ω3). ALA is a precursor fatty acid for the biosynthesis of the LC omega-3 and in particular eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic (DHA, 22:6ω3) fatty acids which are dietary indispensable fatty acids for humans (Howe *et al.*, 2006).

In recent studies, it has been suggested that the omega-3 elongation enzyme cascade could be controlled by genetic factors such as lipid synthesis and fatty acid metabolism-related genes (Mannen, 2011; Knight *et al.*, 2014).

The delta-5 and delta-6 desaturases are key enzymes in LC-PUFA metabolism and several factors including the fatty acid profile in the diet and the type of biological tissues may influence desaturase activity (Zietemann *et al.*, 2010). However, these findings were not observed in ruminant livestock and in recent years a number of studies have therefore been undertaken to understand the relationships between genetics and intramuscular long-chain omega-3 content in ruminants.

Fatty acid binding proteins (FABPs) are proteins that reversibly bind fatty acids and other lipids. Nine tissue-specific cytoplasmic FABPs have been identified (Ordovas, 2007). Fatty acid binding protein 4 (FABP4), which is expressed in adipose tissue, interacts with peroxisome proliferator-activated receptors and binds to hormone-sensitive lipase. It therefore plays an important role in lipid metabolism and homeostasis in adipocytes (Michal *et al.*, 2006). Genetic variability at the FABP4 locus has been shown to be associated with plasma lipid levels, type-2 diabetes, and coronary heart disease risk (Ordovas, 2007). Therefore, FABP4 is a candidate gene

affecting fatness traits of mammals, but Barendse *et al*. (2009) reported that its association with fatness traits in cattle and other livestock species is not consistent from one study to another. For instance, genetic polymorphisms of the FABP4 gene were significantly associated with marbling and carcass weight (Lee *et al.*, 2010) and back fat thickness (Cho *et al.*, 2008) in Korean Hanwoo cattle, but only with palmitoleic acid in Japanese Black cattle (Hoashi *et al.*, 2008).

The FADS2 enzyme acts as the catalyst for the desaturation of ALA to stearidonic acid (SDA, 18:4ω3) and linoleic acid (LA) to γ-linolenic acid (GLA, 18:3ω6). This is followed by an elongation step, after which the FADS1 catalyses the conversion of eicosatetraenoic acid (ETA, 20:4ω3) and dihomo-γ-linolenic acid (DGLA, 20:3ω6) into EPA and arachidonic acid (AA, 20:4ω6), respectively (Nakamura and Nara, 2004).

This experiment set out to use a single gene association approach to test for associations between two putative SNP in the FABP4 region and one SNP at the FADS2 genome region to begin filling in the knowledge gap that had previously existed. No genetic trials designed to develop molecular marker as tools to enhance long-chain omega-3 content in lamb had been undertaken at the time of this trial. The experiment was conducted on a moderate scale data set with very comprehensive fatty acid profiling performed and using genotype data that had been genetically matched. Since this experiment was performed commencing in 2008 a significant amount of resources has been invested by the Australian Sheep CRC to replicate this fatty acid experiment on a larger scale. The CRC used the OvisSNP50 chip to test a dataset of the Information Nucleus Flock (INF) for associations in the FADS and ELOV gene regions and reported no associations for LC omega-3 (Knight *et al.*, 2012). In 2014 the same research group, working with a much larger dataset

and the refined Meat Quality Research SNP chip, found 4 genes that were not occurring in the FADS or ELOV regions that did improve the prediction of LC omega-3 content in lamb, but offered only very small gains in the claimable EPA + DHA content (Knight *et al.*, 2014).

This experiment hypothesised that a small panel of SNP markers could be used to accurately predict the LC omega-3 content in the short loin of Australian lamb using genetically matched muscle and blood samples.

Materials and methods

Animals and experimental design

A half-sib experimental design was utilised in this study. Five top-EBV rams acquired from Tasmanian Sheep Stud Breeders comprising Dorset, Texel, White Suffolk, East Friesian and Coopworth were mated to purebred Merino ewes at a ratio of 1:120 ewes in separate paddocks in a commercial farming operation in the Coal River Valley, Tasmania, to generate 500 first cross prime lambs.

Animal management

Lambs were run in sire groups under similar management conditions to minimise environmental variation. The lambs were marked, vaccinated and electronically tagged at 6 weeks and run as one mob within a large scale commercial farming operation. The flock was raised during a difficult season of severe drought. From the third trimester onwards animals were raised on a mixture of limited irrigated, drought -affected pastures and supplemented with barley. A subset of 40 animals was supplemented with canola and lupin meals over a 45-day feeding trial. Due to drought, the flock was relocated to non-drought affected winter forage crops of oats

and fescue as basal diet. Blood sampling was by jugular venepuncture from 472 prime lambs directly into vacutainers containing EDTA. The prime lambs were slaughtered at the Longford commercial abattoir at 44 kg or greater slaughter weight and carcasses were chilled overnight. *Longissimus dorsi* muscle tissue samples from 369 of the prime lambs were collected and transported to the laboratory in ice-containing baths and stored at -20°C until ready for genomic DNA extraction.

Fatty acid quantification

362 tissue samples of approximately 1 g, cut across the grain of the *longissimus dorsi* muscle at the 12th rib was used for fatty acid analysis. Lipid was extracted using a modified Bligh and Dyer protocol (Bligh and Dyer, 1959). This involved a single phase extraction, $CHCl_3$/MeOH/H_2O (1:2:0.8, by vol.) followed by a phase separation to yield a total lipid extract (TLE). An aliquot of TLE was trans-methylated in methanol: chloroform; hydrochloric acid (10:1:1, v/v/v) for 2 hour at 80°C. After addition of water, the mixture was extracted three times with hexane: dichloromethane (4:1, v/v) to obtain fatty acid methyl esters (FAME) which are concentrated under a stream of nitrogen gas. Samples were made up to a known volume with an internal injection standard (19:0 FAME) and analysed by gas chromatography (GC) using an Agilent technologies 7890A GC (Palo Alto, California, USA) equipped with an Equity-1 fused silica capillary column (15 m×0.1 mm). Helium was used as the carrier gas. Samples were injected, by a split/splitless injector and using an Agilent Technologies 7683B Series auto sampler in splitless mode, at an oven temperature of 120 °C. After 1 min, the oven temperature was raised to 270 °C at 10 °C per min and finally to 300 °C at 5 °C min^{-1} which was held for 5 min. Peaks were quantified with Agilent Technologies GC ChemStation software (Palo Alto, CA, USA). Individual component identification was confirmed by mass spectral data and

by comparing retention time data with those obtained for authentic and laboratory standards. GC–mass spectrometric analyses were performed on a Finnigan Thermoquest GCQ GC–mass spectrometer fitted with an on-column injector and using Thermoquest Xcalibur software (Austin, TX, USA). The GC was fitted with a capillary column of similar polarity to that described above. GC peak areas were converted to mg / 100 g using the 19:0 FAME internal injection standard prior to statistical analysis.

DNA extraction

Genomic DNA from blood samples was extracted using the UltraClean® -htp 96 Well BloodSpin® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA). Muscle samples were extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). DNA concentration of all samples was assessed using the NanoDrop 8000 UV/VIS spectrometer (Thermo Scientific, Wilmington, DE, USA) along with purity by determination of the 260/280 nm ratio.

Analysis of Polymorphism in FABP4, and FADS2

To identify SNP, the International Sheep Genomics Consortium 454 read archive (https://isgcdata.agresearch.co.nz/) was BLAST searched using bovine mRNA sequences of FABP4 (NM_001114667) and FADS2 (NM_001083444). A total of 19 (FABP4) and 50 (FADS2) 454 reads were retrieved and aligned using Sequencer v4.7 before putative SNP were identified as base changes between overlapping reads derived from different sheep. Three variant positions were identified and used to develop fluorogenic 5’ nuclease assays formatted for analysis using the Applied Biosystems (AB) 7900HT “TaqMan’ sequence detection system. FABP4_SNP1 and FABP4_SNP2 are in intron 2 of the FABP4 gene located on sheep chromosome 9 at Mb position 60.52 as defined by sheep genome v1.0

(https://www.biolives.csiro.au/cgi-bin/gbrowse/oar1.0/). FADS2_SNP1 is located within the 3' UTR of the FADS2 gene located on sheep chromosome 21 at Mb position 43.71. The primers and probes used to for genotyping animals at each of the SNP are provided in Table 5.1. Reactions were conducted in 10 µl containing 5 ng of genomic DNA and standard conditions. End point allele discrimination analysis to assign the genotype of each animal was performed using the Sequence Detection System (SDS) software version 2.2 (Applied Biosystems).

Table 5.1 Putative SNP sequences used in omega-3 fatty acid associations.

Gene	SNP	Oligonucleotide Name	Oligonucleotide Sequence
FABP4	FABP4_SNP1	forward primer	GACAGGAAAGTCAAGGTGAGGAATA
	FABP4_SNP1	reverse primer	CCTCCTTCTACAAAATGGCTTGCTA
	FABP4_SNP1	vic-probe	AGAGTAAAAGCCTGATTTATA
	FABP4_SNP1	fam-probe	AGTAAAAGCCTGGTTTATA
	FABP4_SNP2	forward primer	GAGGAATAAAGAACTGGAGCAGAGT
	FABP4_SNP2	reverse primer	CCTCCTTCTACAAAATGGCTTGCTA
	FABP4_SNP2	vic-probe	ATAGGCAGCAGTCGTTTA
	FABP4_SNP2	fam-probe	TAGGCAGCAGTTGTTTA
FADS2	FADS2_SNP1	forward primer	CCCCTGACCTGGCCATT
	FADS2_SNP2	reverse primer	CCAAGTCCAGAGCCTGTGA
	FADS2_SNP3	vic-probe	AGAGCTCAGCAGAAGC
	FADS2_SNP4	fam-probe	AAGAGCTCAACAGAAGC

Parentage Assignment

A set of 31 SNP were used to establish the paternity of progeny developed under an animal management scheme that included use of a backup ram. The SNP were taken from a larger set (Kijas *et al.*, 2009) and the identifier, GenBank accession, sequence context and minor allele frequency for each marker is given in Table 5.1. Blood derived genomic DNA from 485 progeny and five sires was genotyping using a

single Sequenom MassARRAY multiplex at the Australian Genome Research Facility. In addition, a mutation in the *Myostatin* gene responsible for muscle hypertrophy (g+*6723G>A*) was genotyped in all animals using previously described methods (Kijas *et al.*, 2007). For progeny within sire group, genotypes were used to determine paternity between the intended ram and the backup ram. Progeny and ram genotypes (32 SNP) were analysed using Cervus 2 (Marshall *et al.*, 1998). Progeny with discordant genotypes at two or more of the 32 SNP when compared against the intended ram had paternity assigned to the backup ram.

Sample Matching

To ensure fatty acid quantification derived from muscle was correctly matched to SNP alleles derived using blood originating from the same individual, SNP were used for sample matching as follows. DNA derived from 242 tissue samples used for fatty acid quantification were genotyped for the 32 SNP used for paternity assignment. Similarly, blood derived samples from all of the progeny (n = 421) were genotyped using the same set of SNP. The genetic similarity, estimated as allele sharing, was computed between each pair-wise combination of tissue and blood derived DNA sample. Allele sharing was calculated using PLINK v1.07 (Purcell *et al.*, 2007) which reports the average proportion of allele sharing as *D*st. Tissue samples were assigned to their animal or origin where *D*st > 0.95.

Statistical analysis

Statistical analysis for associations between the FABP and FADS2 SNP was modelled using the statistical package R (R Development Core Team, 2013) using the following model.

$$\boldsymbol{y_i = \mu + x_i + s_i + b_i + \varepsilon_i}$$

Where y_i is the phenotype for individual i, μ is the mean (intercept), x_i, s_i and b_i are the SNP genotype, sex and breed of individual i respectively. ε_i is the residual error for individual i. Estimates were estimated using restricted maximum likelihood using a linear mixed model, with SNP and sex fitted as fixed effects and breed as a random effect.

Results

Parentage and ID assignment

The use of the 31 SNP listed in Table 5.2 and Myostatin gene (g+*6723G>A*) showed a high level of accuracy in the prediction of the sire within each treatment group using visual appraisal and a black faced backup ram.

The allele matrix approach to matching tissue derived DNA samples with blood derived DNA samples successfully matched and confirmed the identity of 236 samples. Only 12 blood samples could not be matched to a tissue sample, indicating that only a small number of sampling and recording errors had occurred and these have been addressed using the allele matrix approach. Combining the parentage data and allele matrix data produced a total of 236 progeny that had accurately assigned parentage, phenotypic field measurements, meat quality data and fatty acid profiles.

Table 5.2 SNP used for parentage assignment and verification of tissue samples

ISGC SNP ID[1]	GenBank accession	SNP with flanking sequence	SNP Type	Call Rate[2]	Minor Allele Frequency
CL635944_160	CL635944	GTGAC-[A/G]-GTATT	G/A	92.4	0.40
CZ920359_258	CZ920359	GCCCA-[T/C]-ACCCT	T/C	94.7	0.36
CZ920950_468	CZ920950	CCGAT-[A/G]-AAGAC	G/A	95.1	0.48
DU178311_404	DU178311	TTTTC-[C/T]-AAAGA	C/T	94.0	0.45
DU183112_480	DU183112	CAACC-[T/C]-TTACC	T/C	96.2	0.44
DU191809_420	DU191809	CTCCT-[A/G]-GAAGC	A/G	94.0	0.41
DU200069_211	DU200069	ATTCA-[C/T]-TGAGC	C/T	93.6	0.42
DU202116_405	DU202116	CAATG-[C/T]-TAGTT	C/T	94.4	0.21
DU202534_254	DU202534	AAAGC-[A/G]-GTAAC	A/G	93.0	0.40
DU213735_493	DU213735	GTGCC-[A/G]-TCAAG	A/G	94.8	0.49
DU223430_259	DU223430	ACACC-[T/A]-TAGTG	T/A	90.8	0.49
DU231007_156	DU231007	TTAAC-[T/C]-CACAG	T/C	94.5	0.45
DU231335_636	DU231335	CATCT-[A/G]-CTTTC	A/G	93.6	0.43
DU245518_579	DU245518	GGCTC-[A/G]-GGAAA	G/A	93.5	0.36
DU269694_582	DU269694	AGAAA-[A/G]-AGAAA	A/G	90.8	0.35
DU271929_382	DU271929	AGGAC-[A/C]-GGTTG	A/C	94.3	0.47
DU310703_497	DU310703	ATGAC-[A/G]-AGGTC	A/G	85.8	0.48
DU322055_258	DU322055	TTGCA-[C/T]-ATGGA	C/T	94.0	0.49
DU357209_487	DU357209	GGCCG-[T/G]-AGTTG	T/G	93.9	0.47
DU398082_567	DU398082	GGTGT-[A/G]-TTTAT	A/G	93.4	0.4
DU459528_301	DU459528	CGGGG-[A/T]-GATCA	A/T	91.1	0.39
DU463400_341	DU463400	TTCTT-[T/C]-ATCTC	T/C	92.2	0.44
DU463771_520	DU463771	ACCCA-[T/C]-GTATT	T/C	95.1	0.46
DU464373_638	DU464373	CCAAA-[A/G]-GTAAT	A/G	94.0	0.4
DU469454_586	DU469454	GGCAG-[T/C]-TGTGT	T/C	94.9	0.33
DU470132_375	DU470132	GAGGG-[G/C]-CCAGT	G/C	94.9	0.49
DU492158_335	DU492158	TGGAT-[T/C]-TCTTC	T/C	94.4	0.35
DU492501_194	DU492501	GATGA-[T/G]-ATGCA	T/G	60.3	0.48
DU492723_242	DU492723	GGCTC-[A/G]-TGCTC	A/G	94.0	0.46
DU494996_198	DU494996	GCACA-[C/T]-GTGTA	C/T	94.1	0.37
DU529574_332	DU529574	TTTTC-[G/T]-GACTT	G/T	94.1	0.47

[1] The SNP were taken from Kijas et al., (2009) (PLoS ONE 4: e4668). The genomic location and sequence details for each SNP can be found using the sheep genome browser at http://www.livestockgenomics.csiro.au/cgi-bin/gbrowse/oar1.0/

[2] Call rate is given as a percentage and was calculated from genotyping 768 samples in a single Sequenom multiplex assay.

Table 5.3 Abbreviated table demonstrating assignment of sample matching using average proportion of allele sharing between tissue and blood samples where Dst > 0.95 is denoted in green and a complete allele match (100%) is denoted in red as a value of 1.

Blood DNA Sample Identity	Tissue DNA Sample Identity									
	153	***154***	***155***	***156***	***157***	***158***	***159***	***160***	***161***	***162***
B1	0.633	0.714	0.650	0.607	0.600	0.638	0.696	0.600	0.696	0.696
B10	0.661	1.000	0.565	0.707	0.673	0.683	0.638	0.629	0.652	0.724
B100	0.645	0.690	0.548	0.621	0.808	0.633	0.759	0.710	0.783	0.776
B11	0.554	0.673	0.643	0.714	0.740	0.643	0.796	0.607	0.690	0.731
B12	0.548	0.707	0.677	0.690	0.558	0.717	0.724	0.613	0.717	0.759
B13	0.638	0.759	0.690	0.714	0.600	0.690	0.714	0.707	0.818	0.778
B14	0.574	0.640	0.593	0.673	0.625	0.611	0.981	0.685	0.690	0.700
B15	0.690	0.778	0.690	0.661	0.620	0.707	0.732	0.655	0.762	0.759
B17	0.710	0.741	0.645	0.690	0.712	0.667	0.741	0.677	0.761	0.776

Fatty Acid Profiles

Intramuscular fatty acid content was as varied within breeds as it was between breeds. The drought conditions had a negative impact on the LC omega-3 content. Some animals recorded almost no LC omega-3 content, yet the maximum content observed (Table 5.4) comfortably met the claimable “dietary source” level of 30 mg/100 g (of LC omega-3, specifically EPA+DHA). The contribution of docosapentaenoic acid (DPA, 22:5ω3) is not presently included in this omega-3 claim.

Table 5.4 Summary of intramuscular n-3 LC-PUFA content in lamb longissimus dorsi muscle expressed in mg/100 g of raw tissue.

	EPA	**DHA**	**EPA + DHA**	**EPA + DPA + DHA**
Min	1.1	0.0	0.9	2.1
Max	33.6	14.2	45.6	79.5
Mean	10.4	3.2	14.6	23.2
SD	5.0	2.1	7.4	11.3

The distribution of EPA and DHA plus the summation of EPA+DHA and EPA+DPA+DHA intramuscular FA contents were not normally distributed, with *p*-values from a Shapiro-Wilks test of $2.4x10^{-10}$, $1.7x10^{-9}$ and $4.1x10^{-08}$ respectively. The standard deviations of these content values confirms the high levels of variability observed in this data set. Although ω3DPA is not FSANZ claimable it is an intermediary fatty acid between EPA and DHA and when included in the total LC omega-3 it generally increases the total content of these key ingredients by up to a factor of two for Australian lamb.

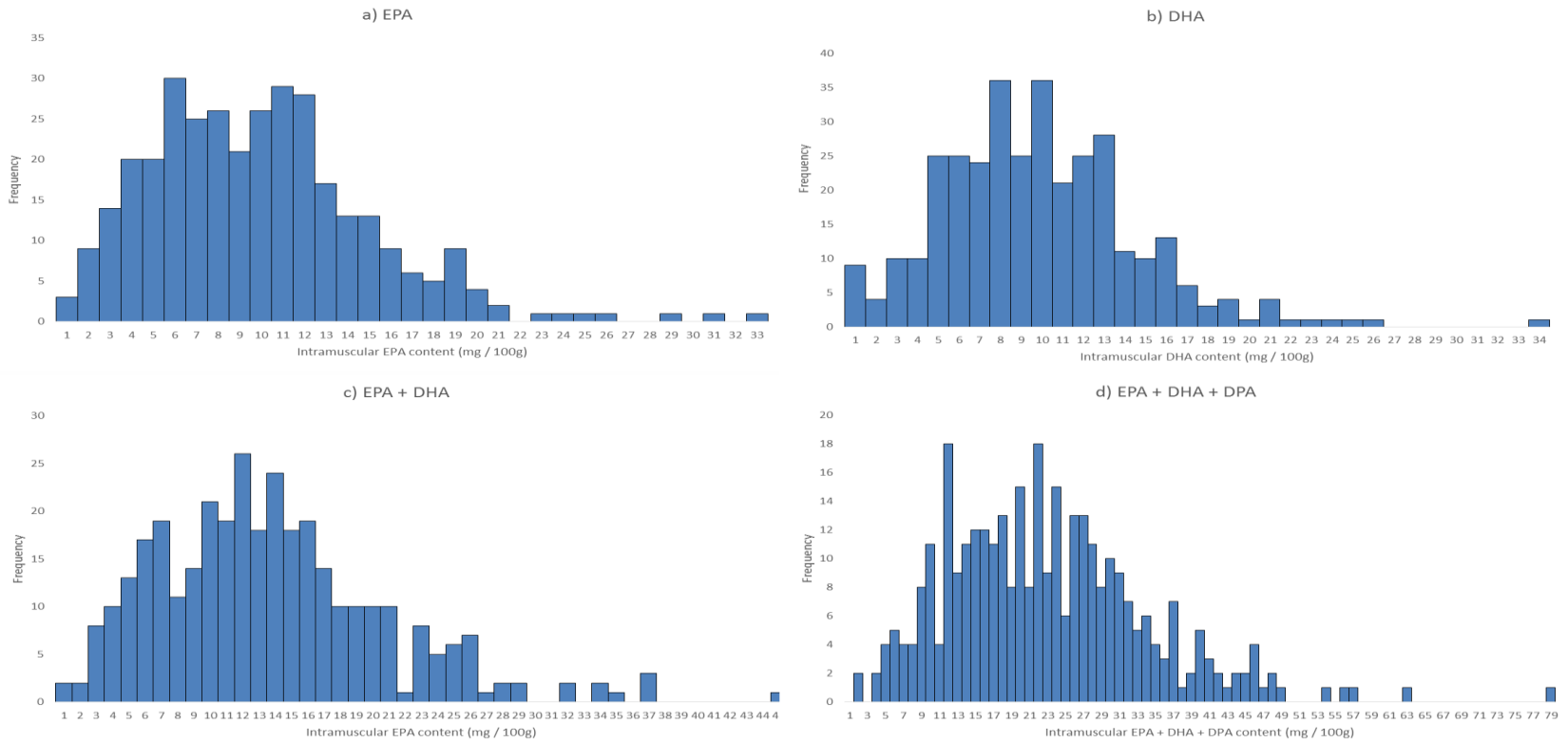

Figure 5.1 a-d. Distribution of key LC-PUFA (mg / 100 g) in the longissimus dorsi of prime lambs. a) Distribution of EPA content for all animals shows that one animal (East Friesian 39) reached good dietary source. b) DHA contents were similarly distributed to EPA, with (EF 39) being significantly higher in DHA than all other animals. c) EPA + DHA contents were distributed predominately around the 10-15 mg/100 g, and 9 animals reached the claimable "source" level of 30mg / 100 g. d) The inclusion of DPA to the EPA + DHA content increased the mean LC omega-3 content by up to twofold.

SNP Fatty Acid Associations

No associations were observed between the SNP in the FABP or FADS gene regions and the long-chain omega-3 fatty acids EPA and DHA at significant levels less than P <0.05 (Table 5.5). No associations occurred between the SNP tested and combined fatty acids EPA + DHA and EPA + DPA + DHA in the *longissimus dorsi* muscle at significant levels less than P <0.05. (Table 5.5). The second FAPB4 only had one AA genotype call and was discounted from the results and when analysed show no significant (P<0.05) associations.

Table 5.5 Summary of SNP association p-values with key intramuscular fatty acids.

	FABP4	FADS2
EPA	0.966	0.573
DHA	0.730	0.682
EPA+DHA	0.887	0.595
EPA+DPA+DHA	0.925	0.578

Discussion

Malerba *et al.* (2008) genotyped 13 SNPs located on the FADS1/2/3 gene cluster in serum phospholipids of humans, and found strong associations with ARA, LA, ALA and eicosadienoic acid, but not SDA, EPA and DHA fatty acids content. Lattka *et al*. (2010) confirmed that polymorphisms of the Delta-5 (FADS1) and Delta-6 (FADS2) desaturase genes was associated with the content of several long-chain ω3 and ω6 PUFAs in serum phospholipids. In the plasma also, genetic variability in the FADS1-FADS2 gene cluster revealed that several SNP were associated with higher delta-6 (FADS2) desaturase activity and lower delta-5 (FADS1) desaturase activity (Bokor *et al.*, 2010). All of these studies used human data sets and at the time when this trial was performed published data from ruminant based trials was minimal.

Barendse *et al.* (2009) genotyped 1409 cattle with a mutation in the FABP4 (FABP4 g.-25et02C) site of the cattle genome representing seven breeds; the authors found an effect of 0.3% of the intramuscular fat content variation, but did not specifically focus on LC omega-3. Inconsistent results are widely reported in the literature, with weak to no interactions observed and more recent studies with the FAB4 site have reported no linkage with IMF or marbling scores (Matsumoto *et al.*, 2014).

In recent years the marketing potential for enhanced or dietary “source” content (30 mg/100 g) of LC omega-3 has been recognized. Increased market competition for fish oil as the primary source of EPA and DHA rich oils has also occurred and led to market premiums being observed globally for red and other meats with elevated LC omega-3 content (Kitessa *et al.*, 2014). The growing consumer interest lead the Sheep CRC to invest significant resources into the inclusion of molecular markers for the prediction of LC omega-3 content in lamb on the recently developed

OvineSNP50 chip. Knight *et al.* (2012) used the recently developed OvineSNP50 chip and the Australian Sheep CRC Information Nucleus Flock dataset to focus on the biosynthesis pathway of LC omega-3 fatty acid synthesis. The FADS 1/2/3, ELOV2 and SCL26A10 loci were investigated with 74 SNP. Results showed no significant ($P < 0.05$) associations between the FADS or ELOV regions despite human trials suggesting strong linkage (Knight *et al.*, 2012).

The most recent and largest data set searching for a genetic association for EPA and DHA content used the OvineSNP50 genotypes to impute 192 SNP for the Sheep CRC Meat Quality Research (MQR) panel using nearly 6200 animals (Knight *et al.*, 2014). The study found 4 significant SNP that when summed could boost EPA + DHA + DPA content by 5.3 mg/100 g and only 2.3 mg/100 g for the claimable EPA + DHA. Interestingly, none of the significant SNP were in the FADS or ELOV genome region and came from unrelated regions of the sheep genome on chromosome 3 (Knight *et al.*, 2014).

The results of the present experiment are in agreement with the more recent genetic work undertaken to identify SNP in the FADS2 and FABP4 regions as breeding tools to enhance LC omega-3 content and suggests the ability to enhance LC omega-3 content is not being limited by genetic diversity, but rather more significantly by the quality of feed on offer. The extreme levels of variation observed would suggest otherwise and high variation is still observed in experimental populations despite a lifetime on irrigated green grass (Kitessa *et al.*, 2010) and drought may just amplify the variation.

The LC omega-3 intramuscular content of the animals in this trial is much lower than observed levels in comparison to a number of recent studies using pasture based systems. However, similar contents were observed in comparison to animals reared

in semi-arid regions of Australia such as inland South Australia (Ponnampalam *et al.*, 2014). Aurousseau *et al.* (2007) observed mean EPA + DHA of 36.7 mg/100 g for lamb grazing green pastures and Kitessa *et al.* (2010) also observed mean EPA + DHA contents that met dietary source levels at sites where green grass was abundant. The observed mean of 14 mg/100 g in this experiment is perhaps attributed to lower levels of the EPA and DHA precursor ALA in the pastures due to drought stress, and also increased omega-6 intake from the use of grain supplementation in the trial. Despite this limiting factor there was still significant diversity in the observed contents of LC omega-3 and it is somewhat unexpected there is not a genetic mutation controlling the high levels of variation observed.

The impact of feed on offer is most likely driving this observation, but individual animal grazing behaviour is another important factor to consider. Sheep grazing in extensive pasture systems exhibit individualistic behaviour, despite being a herd animal, and the inbuilt preference to source a variety of fodder to meet metabolic demands means every animal has a different spectrum of intakes at any point in time (Kyriazakis and Oldham, 1993). This same behaviour was exhibited in the feeding trial component of this experiment; despite animals having the same quantity of feed on offer, intake levels for individual animals varied significantly and may explain more of the variation of IMF fatty acid content that was previously attributed to the FA profiles of the feed on offer (Bignell *et al.*, 2011).

The experimental design of using a black faced backup sire after six weeks with the target sire has been validated as a very effective tool for breeding larger scale half sib sheep populations for research within extensive grazing operations. The visual sire ID call rate was 98.8% accurate; however, this system does rely heavily on the operator calling sire ID to be very knowledgeable of sheep breeds and noticing

subtle breed variations. This process appears novel in the literature with most experiments relying on expensive artificial insemination, resulting in high percentages of unjoined ewes or smaller scale confined joining systems which all incur significant experimental costs. The use of molecular markers to assign parentage is not novel to livestock experiments, however, it is a costly process and more often than not, molecular work is not undertaken till the conclusion of the experiment which can be up to two years from when the sire and dam were joined. Animals with incorrect pedigree are excluded from analysis and ultimately represent an incurred experimental cost for limited data and any method to reduce this occurring is advantageous to the experimental design. This approach also offers a robust data culling method when the animals are marked and again when weaned.

The impediment of successfully matching phenotypic and pedigree information to traits that are measured post slaughter in livestock experiments is a significant source of error and cost in many trials resulting in high percentages of data being culled or inadvertently included. The use of the allele matrix approach to matching unknown genetic material has been used infrequently in plant breeding trials to match samples for shared parentage in large data sets and wildlife populations to determine genetic pedigree (Korir *et al.*, 2013).

Few examples appear in the literature for use of the allele matrix approach as a tool to match genetic samples of unknown identity to known identity in livestock experiments. In this study, the matrix successfully matched 236 tissue samples to their respective blood samples which matched all traits measured pre-slaughter to the correct carcass. Despite a very conscious effort at the kill chute and subsequent short loin collection at carcass break downs, there were errors in the system. The advantages of using the small and relatively inexpensive 32 SNP panel were

significant; it allowed the data to have its pedigree checked accurately and all sample ID's correctly matched to the appropriate animal before testing with putative SNP or conducting more complex genome wide association studies.

Conclusion

A small 32 SNP panel was used to confirm sire breeds and later match blood samples with tissue samples. This approach proved to be a very valuable tool in this scale of experiment which relied on fitting within the constraints of a commercial extensive livestock operation and not owning the carcasses post slaughter. The lack of associations in the FADS2 or FAB4 mutations is consistent with work later conducted by other researchers on a much larger data set and hints towards the impact of genetics controlling LC omega-3 content being a minor component of the overall picture. The impact of drought led to the majority of animals having very low contents of EPA + DHA (expressed on a mg/100 g basis), but some animals still reached dietary "source" levels of 30 mg/100 g or higher for EPA + DHA. Future work into the individual intakes of lambs grazing on green pastures and also monitoring of pasture ALA levels and the subsequent content of intramuscular EPA + DHA may lead to a better understanding of the sources of LC omega-3 content variation in Australian lamb.

References

ABARE (2009).The value of the red meat industry to Australia. In *ABARE Research report*(Eds S. Fletcher, B. Buetre and K. Morey). Australian Government -

Barendse, W., Bunch, R. J., Thomas, M. B. & Harrison, B. E. (2009). A splice site single nucleotide polymorphism of the fatty acid binding protein 4 gene appears to be associated with intramuscular fat deposition in longissimus muscle in Australian cattle. *Animal Genetics* 40(5): 770-773.

Bignell, C. W., Nichols, P. D., Malau-Aduli, A. E. O., Kijas, J. W. & R, M. (2011).Intramuscular long-chain omega 3 content in Australian lamb: Drought effect, genomics and dieatary improvment stratergies. In *Australasian Section of the American Oil Chemists Society*Adelaide.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37(8): 911-917.

Bokor, S., Dumont, J., Spinneker, A., Gonzalez-Gross, M., Nova, E., Widhalm, K., Moschonis, G., Stehle, P., Amouyel, P., De Henauw, S., Denes, M., Moreno, L. A., Meirhaeghe, A., Dallongeville, J. & Grp, H. S. (2010). Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-6 desaturase activities estimated by serum fatty acid ratios. *Journal of Lipid Research* 51(8): 2325-2333.

Cho, S., Park, T. S., Yoon, D. H., Cheong, H. S., Namgoong, S., Park, B. L., Lee, H. W., Han, C. S., Kim, E. M., Cheong, I. C., Kim, H. & Shin, H. D. (2008). Identification of genetic polymorphisms in FABP3 and FABN and putative association with back fat thickness in Korean native cattle. *Bmb Reports* 41(1): 29-34.

Givens, D. I., Khem, K. E. & Gibbs, R. A. (2006). The role of meat as a source of n-3 polyunsaturated fatty acids in the human diet. *Meat Science* 74(1): 209-218.

Hoashi, S., Hinenoya, T., Tanaka, A., Ohsaki, H., Sasazaki, S., Taniguchi, M., Oyama, K., Mukai, F. & Mannen, H. (2008). Association between fatty acid compositions and genotypes of FABP4 and LXR-alpha in Japanese Black cattle. *Bmc Genetics* 9: 7.

Holloway, G. J., Peyton, L. J. & Griffith, G. R. (2000). Was the Australian meat and live-stock corporation's advertising efficient? *Australian Journal of Agricultural and Resource Economics* 44(1): 55-81.

Howe, P., Meyer, B., Record, S. & Baghurst, K. (2006). Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition* 22(1): 47-53.

Kijas, J. W., McCulloch, R., Edwards, J. E. H., Oddy, V. H., Lee, S. H. & van der Werf, J. (2007). Evidence for multiple alleles effecting muscling and fatness at the Ovine GDF8 locus. *Bmc Genetics* 8: 11.

Kijas, J. W., Townley, D., Dalrymple, B. P., Heaton, M. P., Maddox, J. F., McGrath, A., Wilson, P., Ingersoll, R. G., McCulloch, R., McWilliam, S., Tang, D., McEwan, J., Cockett, N., Oddy, V. H., Nicholas, F. W., Raadsma, H. & International Sheep Genomics, C. (2009). A Genome Wide Survey of SNP Variation Reveals the Genetic Structure of Sheep Breeds. *Plos One* 4(3): 13.

Kitessa, S., Liu, S. M., Briegel, J., Pethick, D., Gardner, G., Ferguson, M., Allingham, P., Nattrass, G., McDonagh, M., Ponnampalam, E. & Hopkins, D. (2010). Effects of intensive or pasture finishing in spring and linseed supplementation in autumn on the omega-3 content of lamb meat and its carcass distribution. *Animal Production Science* 50(2): 130-137.

Kitessa, S. M., Abeywardena, M., Wijesundera, C. & Nichols, P. D. (2014). DHA-Containing Oilseed: A Timely Solution for the Sustainability Issues Surrounding Fish Oil Sources of the Health-Benefitting Long-Chain Omega-3 Oils. *Nutrients* 6(5): 2035-2058.

Knight, M. I., Daetwyler, H. D., Hayes, B. J., Hayden, M. J., Ball, A. J., Pethick, D. W. & McDonagh, M. B. (2012). Discovery and trait association of single nucleotide polymorphisms from gene

regions of influence on meat tenderness and long-chain omega-3 fatty acid content in Australian lamb. *Animal Production Science* 52(6--7): 591-600.

Knight, M. I., Daetwyler, H. D., Hayes, B. J., Hayden, M. J., Ball, A. J., Pethick, D. W. & McDonagh, M. B. (2014). An independent validation association study of carcass quality, shear force, intramuscular fat percentage and omega-3 polyunsaturated fatty acid content with gene markers in Australian lamb. *Meat Science* 96(2): 1025-1033.

Korir, P. C., Zhang, J., Wu, K. J., Zhao, T. J. & Gai, J. Y. (2013). Association mapping combined with linkage analysis for aluminum tolerance among soybean cultivars released in Yellow and Changjiang River Valleys in China. *Theoretical and Applied Genetics* 126(6): 1659-1675.

Kyriazakis, I. & Oldham, J. D. (1993). Diet selection in sheep - the ability of growing lambs to select a diet that meets their crude protein (nitrogen x 6.25) requirements. *British Journal of Nutrition* 69(3): 617-629.

Lattka, E., Illig, T., Koletzko, B. & Heinrich, J. (2010). Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism. *Current Opinion in Lipidology* 21(1): 64-69.

Lee, S. H., van der Werf, J. H. J., Lee, S. H., Park, E. W., Oh, S. J., Gibson, J. P. & Thompson, J. M. (2010). Genetic polymorphisms of the bovine Fatty acid binding protein 4 gene are significantly associated with marbling and carcass weight in Hanwoo (Korean Cattle). *Animal Genetics* 41(4): 442-444.

Malerba, G., Schaeffer, L., Xumerle, L., Klopp, N., Trabetti, E., Biscuola, M., Cavallari, U., Galavotti, R., Martinelli, N., Guarini, P., Girelli, D., Olivieri, O., Corrocher, R., Heinrich, J., Pignatti, P. F. & Illig, T. (2008). SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. *Lipids* 43(4): 289-299.

Mannen, H. (2011). Identification and utilization of genes associated with beef qualities. *Animal Science Journal* 82(1): 1-7.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7(5): 639-655.

Matsumoto, H., Nogi, T., Tabuchi, I., Oyama, K., Mannen, H. & Sasazaki, S. (2014). The SNPs in the promoter regions of the bovine FADS2 and FABP4 genes are associated with beef quality traits. *Livestock Science* 163: 34-40.

Michal, J. J., Zhang, Z. W., Gaskins, C. T. & Jiang, Z. (2006). The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F-2 crosses. *Animal Genetics* 37(4): 400-402.

Nakamura, M. T. & Nara, T. Y. (2004). STRUCTURE, FUNCTION, AND DIETARY REGULATION OF Δ6, Δ5, AND Δ9 DESATURASES. *Annual Review of Nutrition* 24(1): 345-376.

Ordovas, J. M. (2007). Identification of a functional polymorphism at the adipose fatty acid binding protein gene (FABP4) and demonstration of its association with cardiovascular disease: A path to follow. *Nutrition Reviews* 65(3): 130-134.

Piggott, N. E., Chalfant, J. A., Alston, J. M. & Griffith, G. R. (1996). Demand Response to Advertising in the Australian Meat Industry. *American Journal of Agricultural Economics* 78(2): 268-279.

Ponnampalam, E. N., Butler, K. L., Pearce, K. M., Mortimer, S. I., Pethick, D. W., Ball, A. J. & Hopkins, D. L. (2014). Sources of variation of health claimable long chain omega-3 fatty acids in meat from Australian lamb slaughtered at similar weights. *Meat Science* 96(2, Part B): 1095-1103.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J. & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* 81(3): 559-575.

R Development Core Team (2013).R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Rowe, J. B. (2010). The Australian sheep industry - undergoing transformation. *Animal Production Science* 50(11-12): 991-997.

Williams, P. & Droulez, V. (2010). Australian red meat consumption - implications of changes over 20 years on nutrient composition. *Food Australia* 62(3): 87-94.

Zietemann, V., Kroger, J., Enzenbach, C., Jansen, E., Fritsche, A., Weikert, C., Boeing, H. & Schulze, M. B. (2010). Genetic variation of the FADS1 FADS2 gene cluster and n-6 PUFA composition in erythrocyte membranes in the European Prospective Investigation into Cancer and Nutrition-Potsdam study. *British Journal of Nutrition* 104(12): 1748-1759.

Chapter 6

Friesian sheep carry a functional allele at the GDF8 locus

Abstract

The East Friesian is one of the world's most productive sheep milking breeds and its high fecundity, lean meat and mothering abilities has been of interest to sheep breeders to improve meat production. The East Friesian is likely to have contributed to the foundation of other breeds, such as the Texel which originated on the island of Texel which is part of the Netherland's chain of West Friesian islands. The Texel is a meat breed which displays a muscle hypertrophy phenotype caused by a G to A substitution (g+6723G>A) in the growth and differentiation factor 8 gene (GDF8 or Myostatin). Stud sires from across Australia (n=79) were genotyped for g+6723G>A and two microsatellites which flank GDF8 (BM81124 and BULGE20) was performed. GDF8 g+6723G>A mutation in East Friesian revealed 29 animals (37%) were homozygous for the functional allele (A/A), a further 41 (52%) were heterozygous (A/G) and the remaining 9 animals (11%) were wild-type (G/G). The estimated frequency of g+6723A within the East Friesian was 0.63 which is lower than for Texel where it is near fixation. Microsatellites BM81124 and BULGE20 were genotyped revealing the same combination of microsatellite alleles observed in the Texel. This strongly suggests a common origin for the mutation in East Friesian and Texel sheep.

Keywords: East Friesian, GFD8, Myostatin, Texel, Bulge20, Muscle

Introduction

The East Friesian breed of sheep was developed in northern Germany and the Netherlands and has become one of the world's most productive dairy sheep. The breed was introduced into Australia via New Zealand as genetic stock to improve the emerging sheep dairying industries and boost sheep meat production (Allison, 1995). The milking performance of East Friesian was targeted as a trait to transfer into composite prime lamb operations via crossbreeding with meat breeds to increase conception rates, lamb survivability and pre weaning growth rates (Allison, 1995; Thomas *et al.*, 1999). East Friesian breeders noticed a particularly muscular subset of rams was occurring each generation and producers who crossed these muscled sires with composite ewes reported the resulting lambs exhibited higher levels of carcass muscling when compared to progeny from less muscular East Friesian sires.

The introduction of dairy genetics to a composite meat breeding operation was assumed to decrease muscle yield and reduce subcutaneous fat in the progeny, however the impact appeared minimal and instead some gains were observed in animal performance both in Australian flocks and across the globe (Larsgard and Standal, 1999; Thomas *et al.*, 1999; Allison, 1995; Malau-Aduli, 2006). These observations suggested a genetic factor with reasonably high heritability related to meat production was present in the East Friesian genetic makeup.

The East Friesian is likely to have contributed to the foundation of other breeds, such as the Texel which originated on the island of Texel which is part of the Netherland's chain of West Friesian islands. The Texel is a meat breed which displays a muscle hypertrophy phenotype caused by a G to A substitution (g+6723G>A) in the growth

and differentiation factor 8 gene (GDF8 or Myostatin) (Clop *et al.*, 2006). This is one of the few functional mutations which have been elucidated for sheep. Given the likelihood of a common population history linking Texel and East Friesian, we sought to determine if the latter also carries the functional g+6723A GDF8 allele despite the divergent production profiles of the two breeds. This chapter tested the hypothesis that a significant detection of the GDF8 mutation will align shared phylogenetic source of origin with other known sheep breeds.

Materials and methods

A total of 79 pure bred East Friesian sheep were sampled from studs distributed across Eastern Australia which originated from the flock described by Allison (1999). Genotyping of g+6723G>A and two microsatellites which flank GDF8 (BM81124 and BULGE20) was performed as described previously (Kijas *et al.*, 2007).

Results

Parentage and ID assignment

Analysis of the GDF8 g+6723G>A mutation in East Friesian revealed 29 animals (37%) were homozygous for the functional allele (A/A), a further 41 (52%) were heterozygous (A/G) and the remaining 9 animals (11%) were wild-type (G/G). Detection of the g+6723A allele within the East Friesian grouped it with the Texel, White Faced Suffolk, Lincoln and Charollais as breeds known to segregate the mutation at the GDF8 locus (Kijas *et al.*, 2007; Hadjipavlou *et al.*, 2008). The

estimated frequency of g+6723A within the East Friesian was 0.63 which is lower than for Texel where it is near fixation.

Discussion

The estimated frequency of g+6723A within the East Friesian was 0.63 which is lower than for Texel where it is near fixation. This likely reflects the emphasis put on milk production by East Friesian breeders but suggests that selection could quickly produce highly muscled animals which explains the reason behind prime lamb producers exploiting the East Friesian's large body framework for crossbreeding with smaller Merino breeds.

Previous work reported that the g+6723A mutation was found on a single haplotype defined by alleles at two flanking microsatellites (BM81124 allele 218 and BULGE20 allele 141) (Kijas *et al.*, 2007; Clop *et al.*, 2006). To determine if g+6723A in East Friesian is accompanied by the same haplotype, microsatellites BM81124 and BULGE20 were genotyped in ten homozygous (A/A) sheep. The results revealed seven of the animals (70%) were homozygous for the same combination of microsatellite alleles observed in the Texel. This strongly suggests a common origin for the mutation because the alternative scenario, that the mutation has arisen independently within each breed, would predict the allele is accompanied by different haplotypes. The close geographic proximity of the breeds and anecdotal information suggest a shared origin, which explains why the allele appears to be identical by descent in both East Friesian and Texel sheep.

Conclusion

The confirmed presence of g+6723A allele in the Australian East Friesian genetic stock provides another tool for producers to improve their animal performance via genetic screening. The flanking microsatellite alleles strongly suggest a common heritage between the East Friesian and Texel as was anecdotally suggested till now. The potential to use g+6723A as a marker in East Friesians to breed for meat focused animals has great potential. This opens up opportunities to transfer the benefits offered by the dairy traits into other meat breeds and minimise adverse impacts on carcass quality. The impact of GDF8 and its transfer from East Friesian sires into composite lamb progeny and resultant impact on carcass quality is yet to be determined and an area of future investigation.

References

Allison, A. J. (1995). *Importing a sheep which offers more - the East Friesian.* New Zealand Society of Animal Production.

Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibe, B., Bouix, J., Caiment, F., Elsen, J.-M., Eychenne, F., Larzul, C., Laville, E., Meish, F., Milenkovic, D., Tobin, J., Charlier, C. & Georges, M. (2006). A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nat Genet* 38(7): 813-818.

Hadjipavlou, G., Matika, O., Clop, A. & Bishop, S. C. (2008). Two single nucleotide polymorphisms in the myostatin (GDF8) gene have significant association with muscle depth of commercial Charollais sheep. *Animal Genetics* 39(4): 346-353.

Kijas, J. W., McCulloch, R., Edwards, J. E. H., Oddy, V. H., Lee, S. H. & van der Werf, J. (2007). Evidence for multiple alleles effecting muscling and fatness at the Ovine GDF8 locus. *Bmc Genetics* 8: 11.

Larsgard, A. G. & Standal, N. (1999). Introduction of East Friesian dairy sheep into the Norwegian sheep population. *Small Ruminant Research* 33(1): 87-98.

Malau-Aduli, A. B., CW. Tavassoli-Salardini, F. Smolenski, AJ. Palmer, A. Bignell, J. Burbury, S. Batchelor, R. Malau-Aduli, BS. Adediran, SA. Lane, PA. Clark, RJ. (2006).Genetic diversity and breed comparison of carcass traits in Tasmanian Corriedale and East-Friesian sheep by RAPD markers. In *Harnessing and Exploiting Global Opportunities', Proceedings of the 52nd International Congress of Meat Science and Technology*, 75-76 (Ed R. P. D. Troy, B. Byrne, J. Kerry). University College Dublin, Ireland: Wageningen Academic Publishers.

Thomas, D. L., Berger, Y. M. & McKusick, B. C. (1999).Milk and lamb production of East Friesian-cross ewes in the north central United States. In *Milking and Milk Production of Dairy Sheep and Goats*, 474-477 (Eds F. Barillet, S. Deligeorgis, N. P. Zervas, J. G. Boyazoglu and F. Vallerand). Wageningen: Wageningen Academic Publishers.

Chapter 7

General conclusions and future research directions for enhancing long-chain omega-3 content in lamb

The shift in consumer demand for healthy, leaner red meat has seen lamb maintain its market share in the Australian domestic market (MLA, 2014). Sheep meat and lamb in particular, is perceived as a healthy red meat due to the heavy trimming of cuts to remove subcutaneous fat and in addition enhanced consumer understanding of pasture reared animals having healthier fats (Williams and Droulez, 2010). The increased understanding of the health benefits from a diet rich in omega-3 fatty acids is now well understood and the sheep meat industry has invested heavily in promoting the associated health benefits of consuming lamb and its place as a dietary source of long-chain omega-3 (LC omega-3) in the Australian diet (Clayton, 2014). The results of this thesis, other research undertaken by the University of Tasmania team (Flakemore, 2014a; Holman, 2014), and also separate research by the Sheep CRC team (Pannier *et al.*, 2010) indicate, however, to the best of my knowledge that current Australian lamb largely does not consistently meet the current dietary source guidelines.

This thesis investigated the potential to enhance long-chain omega-3 in five common Australian sheep meat breeds and understand the impacts breed, sex, supplementation and the potential for discovery of single nucleotide polymorphic (SNP) marker(s) to explain long-chain omega-3 content variation observed in sheep meat. In addition, the climate circumstances that occurred during the farm trial conducted in this study meant that the effects of a particularly strong one in 200 year drought were also examined during the study.

Chapter 2 tested the hypothesis that finishing lambs with the ALA-containing supplements - canola meal and lupin - for 9 weeks could remediate the negative effects of drought on long-chain omega-3 content in Australian lamb. In the feeding trial experiments, it was found that supplementation with canola meal and cracked lupins did not increase the long-chain omega-3 content to meet the FSANZ claimable source of 30 mg/100 g. Supplementation with shorter chain omega-3 fatty acid rich feeds such as linseed, has been demonstrated to help meet claimable source levels, although it was based on the calculation using a 135 g wet serve of meat and the animals were grazing pastures with a declining plane of nutrition prior to supplementation (Kitessa *et al.*, 2010). In contrast, the animals herein had spent the majority of their lives on stressed pastures and the base level of long-chain omega-3 in the *L. dorsi* muscle was very low to begin with. The impact of drought is clearly very negative on long-chain omega-3 content and future research should focus on finding more effective methods of supplementation to promote both efficient growth and enhanced long-chain omega-3 uptake and or production from precursors in drought situations.

Enhancing the long-chain omega-3 content in sheep meat can introduce negative factors in meat quality which ultimately results in decreased value to the farmer and consumer. Chapter 3 investigated the effects of single nucleotide polymorphic (SNP) loci, sire breed, dietary supplementation with omega-3 polyunsaturated fatty acids and relocation to non-drought affected pastures on the contents of intramuscular long-chain omega-3 and meat quality. The experiment slaughtered animals based on growth rates and, it was observed that if animals met the required >44.5 kg live weight, there were no negative impacts on meat quality. The slaughter protocol also allowed for clearer genetic distinctions. Breed proved to have an impact on fat

scores with East Friesians being significantly leaner and the Dorset and White Suffolk demonstrated a preference towards higher growth and larger, fatter carcasses. Sex did have an effect on the GR Fat score with females being slightly fatter than wethers at the same carcass weight. Various studies have reported significant impact of sex on intramuscular and subcutaneous fat deposition (Dervishi *et al.*, 2012; Flakemore, 2014a; Flakemore, 2014c; Holman, 2014; Pethick *et al.*, 2004). Sexual dimorphism between ewes and wethers in terms of GR fat score is linked to hormonal variation as females generally have the propensity for accelerated early fat deposition compared to males where muscular accretion is more pronounced. Furthermore, the mechanism for the effect of sex on GR Fat score is thought to be dictated by cellular signal transductions and their subsequent impacts on enzymatic pathways linked to lipogenesis. This is an area that needs further research to reveal the underlying biological and molecular mechanisms. None of the tested SNP for omega-3 pathways had a significant effect on meat quality and these findings were discussed more thoroughly in Chapter 5.

The analysis of the fatty acid (FA) composition of the *Longissimus dorsi* muscle gives insight into both the nutritional value and background feeding of animals. Chapter 4 profiled the base-line fatty acids in the five commonly used breeds in Australian lamb operations with a specific focus placed on the long-chain omega-3 fatty acids. The experiment was conducted during a severe drought with erratic rainfall events and unreliable irrigation water which ultimately required relocation of the flock to lush pastures in Northern Tasmania (B.O.M., 2013). Initial pasture sampling and analysis of the FA composition revealed the green and actively growing pastures were rich in the long-chain omega-3 precursor fatty acid - alpha-linolenic acid (ALA) - during spring and early summer. As the experiment

progressed, moisture stress became more evident and less green grass was available and saturated fatty acids became the dominant FA in the pasture sward. This was reflected in the FA profiles of the first two kill groups which had extremely low levels of the long-chain omega-3 fatty acids EPA and DHA. The first two slaughter groups recorded only 7 mg/100 g EPA + DHA which was well below contents reported in other Australian trials (Kitessa *et al.*, 2010; Ponnampalam *et al.*, 2010). This observation clearly demonstrated the negative impact of low ALA content pastures in severe drought stressed situations. To the best of our knowledge, this is the first time the impact of severe drought on the long-chain omega-3 content of Australian lamb has been quantified. The results show future research and associated development are needed to further investigate techniques to mitigate this negative impact as the methods explored in Chapter 2 of this thesis did not satisfactorily increase the EPA + DHA content to meet dietary source levels and animal performance was also not optimal.

When the remaining flock was relocated to a non-drought affected area and abundant green grass was on offer, the third slaughter group showed higher intramuscular fat content and the mean EPA + DHA content was more than double the first two kill groups (15 mg/100 g vs 7 mg/100 g). The results suggest the simplest method is currently relocation of the flock, however, this is not always possible or practicable and the relocated animals still did not reach the claimable dietary source level. This issue was also observed in the Information Nucleus Flock (INF) trials, where only sites with ample green grass then strategic supplementation with an optimised compound ration and ALA rich supplement reached the claimable source level (Kitessa *et al.*, 2010; Pannier *et al.*, 2010).

The observed contents of long-chain omega-3 and in particular EPA and DHA were extremely varied both within and across breeds indicating no existing preference for certain breeds to have propensity for storing intramuscular EPA and DHA. This observation is in keeping with the majority of studies which investigated intramuscular EPA and DHA content (Clayton, 2014).

This high level of variation in EPA and DHA content in lamb *Longissimus dorsi* muscle formed the basis for the research question posed in Chapter 5. It was hypothesised that a small panel of SNP markers could be developed and used to accurately predict the long-chain omega-3 content in the short loin of Australian lamb using genetically matched muscle and blood samples. Three putative SNP were identified and genotyped in the fatty acid binding protein (FABP) and Delta-6 desaturase (FADS2) gene clusters and tested for single gene associations. Mixed model statistical analysis for association with intramuscular EPA and DHA content showed no significant effect. The Sheep CRC also investigated their larger INF dataset in a genome wide association study and also found no significant associations in the FADS gene cluster for EPA or DHA content (Knight *et al.*, 2012). The CRC later developed a Meat Quality Research SNP Chip and found 4 genes that offered small gains in the prediction of long-chain omega-3 content, but these genes were not in the FADS or FABP gene clusters (Knight *et al.*, 2014). Overall it appears that the potential for genetic marker assisted breeding currently has limited gains to offer. Rather the impacts of feed offered are much more marked, although it appears that feed does not help control the high levels of EPA and DHA variation within a flock grazing the same feedstock. Future advances in molecular techniques may unlock greater understanding of desaturase and elongase pathways and continued research into this area holds merit.

The choice of technique used for matching phenotypic data to fatty acid profiles and ultimately genetic genotyping is always a challenge in livestock experiments of any scale and often a source of error despite best practice. The unique approach taken in this experiment of using the relatively inexpensive panel of 32 SNP to match blood derived samples and tissue samples via an allele matrix proved to be a very valuable tool and its use is not a common practice in livestock sciences. Despite all precautions taken, some errors occurred in sample identification and these samples were dismissed from the data set for putative SNP testing thereby improving the quality of the data set taken forward for minimal cost and effort. The allele matrix approach is commonly used in plant breeding trials and some wildlife population studies to determine genetic pedigree and it has proven a very cost effective tool in this half-sib F1 progeny experiment.

A secondary question posed during the molecular marker investigation was if East Friesian shared a common phylogenetic source of origin with other known sheep breeds via sharing of the Myostatin gene (g+6723A). The experiment undertaken in Chapter 6 tested the hypothesis that a significant detection of the GDF8 mutation (g+6723A) will align shared phylogenetic source of origin with other known sheep breeds. The East Friesian and Texel breeds are relatively new to Australia (1990's), however, they share the same geographic origin of the Friesian Islands. Australian East Friesian breeders had noted a more muscular line of animals, which were more suited to meat production than to dairy in their stud flocks. Given the entire East Friesian genetic pool is still reasonably confined in Australia, every genetic line imported to Australia was sampled. The results showed the alleles strongly suggest a common heritage between the East Friesian and Texel breeds which was only anecdotally suggested till now. This observation is a very useful finding for sheep

meat producers who wish to improve their composite sheep breeds by enhancing milk production and mothering instincts without compromising meat quality.

Together the findings of this thesis study demonstrate that long-chain omega-3 content in Australian lamb is highly varied and that the availability of green feed on offer is still currently the greatest determinant of EPA + DHA content in *L. dorsi* muscle. The results demonstrated that when grazing pasture on a declining plane of nutrition resulting from weaning in a drought, the impact on the heathy eating values of the meat if significant lowered. The meat is still considered to be relatively healthy with an attractive omega-3 to omega-6 ratio and therefore represents a balanced part of the Australian diet compared to many other alternative proteins. The lack of association with the tested molecular markers has proven this to be difficult approach not only in this experiment, but also by other larger and better resourced research groups. This is an area which requires significant investment in research and resources to test further. Presently work into optimising and understanding the impacts of feed on offer appears a more rapid and logistically feasible pathway for enhancing long-chain omega-3 content in sheep.

One of the most pertinent areas of research to build upon the discoveries from this study is in greater profiling of the fatty acid profiles in the feeds on offer and also individual feed intake of sheep. Very little work to date has been undertaken to profile the wide range of fatty acid profiles in the commonly used fodders in Australian lamb production systems. It was demonstrated in Chapter 4 that as summer progressed into autumn that the ALA content of the pasture decreased to negligible concentration and resulted in the low EPA + DHA contents in the sheep meat. In severe climatic periods, like those experienced during the trial conducted in this study, supplementation was essential and future research into techniques to

conserve forages rich in ALA would have merit. The use of ALA rich oils or feeds such as linseed in conjunction with a compound ration similar to those used in feedlots was proven by Kitessa (2010) to remediate the impacts of low grade pasture. This technique has to date had limited uptake by the Australian sheep meat industry as feed-lotting is not common practice and techniques utilising hay, silage and demand feeders are more commonly employed.

The effects of these different forage conservation techniques and fodder sources on the levels of conserved ALA to be used as a supplementary feed is largely unknown. Understanding these factors in more depth may provide more efficient and potentially more widely practiced techniques to supplement animals on declining planes of nutrition and still maintain a higher content of long-chain omega-3.

Profiling of the ALA content of various pastures also has strong merit given the relocation of animals to modern tetraploid ryegrass which was lush and green for nearly 4 months at the end of the experiment did not bring the animals to claimable source. A survey of commonly grown pastures and forage crops would help producers maximise the potential for long-chain omega-3 content in their livestock.

Given that red meat is a large source of the Australian dietary EPA + DHA, any method to enhance the nutritional profile of the meat is going to potentially have a positive impact on population health. If producers can maximise their feeding programs to include greater use of green, actively growing grasses and then also use strategic supplementary feeds to remediate periods when this cannot be achieved, the potential to enhance Australian lamb to not only claimable source levels (30 mg/100 g), but also good source levels (60 mg/100 g) appears feasible.

References

B.O.M. (2013).Monthly rainfall Richmond (Lowlands) Vol. 2013: Bureau of Meterology.

Clayton, E. H. (2014).Graham Centre Monograph No. 4: Long-chain omega-3 polyunsaturated fatty acids in ruminant nutrition: benefits to animals and humans. (Eds T. Nugent and C. Nicholls). Wagga Wagga NSW: NSW Department of Primary Industrie.

Dervishi, E., Joy, M., Alvarez-Rodriguez, J., Serrano, M. & Calvo, J. H. (2012). The forage type (grazing versus hay pasture) fed to ewes and the lamb sex affect fatty acid profile and lipogenic gene expression in the longissimus muscle of suckling lambs1. *Journal of Animal Science* 90(1): 54-66.

Flakemore, A. R., Balogun, R.O., McEvoy, P.D., Malau-Aduli, B.S., Nichols, P.D., Malau-Aduli, A.E.O. (2014a). Genetic variation in intramuscular fat of prime lamb supplemented with varying concentrations of degummed crude canola oil. *International Journal of Nutrition and Food Sciences* 3(3): 230-209.

Flakemore, A. R., McEvoy, P.D., Balogun, R.O., Malau-Aduli, B.S., Nichols, P.D., Malau-Aduli, A.E.O. (2014c). Degummed crude canola oil supplementation affects fat depot melting points in purebred and first-cross Merino sheep. *Animal and Veterinary Sciences* 2: 75-80.

Holman, B. W. B., Flakemore, A.R., Kashani, A., Malau-Aduli, A.E.O. (2014). Spirulina supplementation, sire breed, sex and basal diet effects on lamb intramuscular fat percentage and fat melting points. *International Journal of Veterinary Medicine: Research & Reports* 2014: 9.

Kitessa, S., Liu, S. M., Briegel, J., Pethick, D., Gardner, G., Ferguson, M., Allingham, P., Nattrass, G., McDonagh, M., Ponnampalam, E. & Hopkins, D. (2010). Effects of intensive or pasture finishing in spring and linseed supplementation in autumn on the omega-3 content of lamb meat and its carcass distribution. *Animal Production Science* 50(2): 130-137.

Knight, M. I., Daetwyler, H. D., Hayes, B. J., Hayden, M. J., Ball, A. J., Pethick, D. W. & McDonagh, M. B. (2012). Discovery and trait association of single nucleotide polymorphisms from gene regions of influence on meat tenderness and long-chain omega-3 fatty acid content in Australian lamb. *Animal Production Science* 52(6--7): 591-600.

Knight, M. I., Daetwyler, H. D., Hayes, B. J., Hayden, M. J., Ball, A. J., Pethick, D. W. & McDonagh, M. B. (2014). An independent validation association study of carcass quality, shear force, intramuscular fat percentage and omega-3 polyunsaturated fatty acid content with gene markers in Australian lamb. *Meat Science* 96(2): 1025-1033.

MLA (2014).Australian Sheep Meat Industry Overview. In *Australian Sheep Meat Industry Projections 2014*: Meat and Livestock Australia.

Pannier, L., Ponnampalam, E. N., Gardner, G. E., Hopkins, D. L., Ball, A. J., Jacob, R. H., Pearce, K. L. & Pethick, D. W. (2010). Prime Australian lamb supplies key nutrients for human health. *Animal Production Science* 50(11-12): 1115-1122.

Pethick, D. W., Harper, G. S. & Oddy, V. H. (2004). Growth, development and nutritional manipulation of marbling in cattle: a review. *Australian Journal of Experimental Agriculture* 44(7): 705-715.

Ponnampalam, E. N., Warner, R. D., Kitessa, S., McDonagh, M. B., Pethick, D. W., Allen, D. & Hopkins, D. L. (2010). Influence of finishing systems and sampling site on fatty acid composition and retail shelf-life of lamb. *Animal Production Science* 50(8): 775-781.

Williams, P. & Droulez, V. (2010). Australian red meat consumption - implications of changes over 20 years on nutrient composition. *Food Australia* 62(3): 87-94.

Appendix of Publications

These articles have been removed for copyright or proprietary reasons.

Bignell, C.W., Malau-Aduli, A.E.O., Nichols, P.D., McCulloch, R. & Kijas, J.W. (2010). East Friesian sheep carry a Myostatin allele known to cause muscle hypertrophy in other breeds. Animal genetics 41(4): 445-446

Wijesundera, C., Kitessa, S., Abeywardena, M., Bignell, W. & Nichols, P.D. (2011). Long-chain omega-3 oils: current and future supplies, food and feed applications, and stability. Lipid technology 23(3): 55-58

Malau-Aduli, A.E.O., Walker, R.E., Bignell, C.W. (2009). Prediction of wool fibre diameter from protein and metabolisable energy digestibility coefficients in crossbred sheep. J. Anim. Sci., 87 (E-Suppl 2), 498

Malau-Aduli, A.E.O., Ranson, C.F., Bignell, C.W. (2009a). Wool quality and growth traits of Tasmanian pasture-fed crossbred lambs and relationships with plasma metabolites. J. Anim. Sci., 87 (E-Suppl 2), 499